Formulation development of topical antibacterial lotion with *Theobroma cacao* pod husk ash extract for treatment of shaving bumps

**Abstract**

The aims of this study were to determine the emulsifying properties of *Theobroma cacao* pod husk ash (CPHA) methanolic extract combined with shea butter and explore the antibacterial activities and physicochemical characteristics of resulting emulsions toward the development of a topical antibacterial lotion formulation for shaving bumps treatment. The ash resulting from combustion of pod husks of freshly harvested ripe cocoa fruits was extracted with methanol and the extract evaporated to dryness. Shea butter was also extracted by traditional method from kernels from the shea tree. These natural-source materials were combined with pharmaceutical ingredients (buffer, viscosity enhancer, preservative) to develop fluid emulsion formulations. Stability characteristics (droplet size, viscosity, creaming, and pH) of the formulations were evaluated as well as their antibacterial activities against microorganisms isolated from after-shave bump swabs of adult male volunteers and against reference organisms; in order to select product(s) of best qualities suitable as shaving bumps medication. The prototype formulations exhibited suitable physicochemical properties and demonstrated inhibitory activities against several isolated shaving bump microbes and the reference organisms namely, *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. Two formulations were finally selected as having physicochemical and antibacterial qualities most suitable for shaving bumps therapy, which contained shea butter (20%), citrate buffer (5%), and parabens (0.3%), prepared using 5% CPHA extract solution with and without methyl cellulose (2%), respectively. The novel shea butter-incorporated emulsion-lotion formulations of CPHA extract provide a useful therapeutic option of topical medication for the treatment of shaving bumps in men.

**Keywords:** *Theobroma cacao*, cocoa pod husk ash extract, lotion formulation, antibacterial activity, shaving bumps treatment

**Abbreviations:** PFB, pseudofolliculitis barbae; CPHA, *T. cacao* pod husk ash

**Introduction**

Shaving bumps, *Pseudofolliculitis barbae* (PFB), are an inflammatory disorder of follicular and peri-follicular skin resulting from ingrown hairs, which occur most commonly in the beard areas of men, are cosmetically disfiguring and may be psychologically distressing for affected persons. PFB is caused by the shaving of terminal hair in genetically predisposed persons with tight curly hair curved with its concavity toward the epidermis.1 After a close shave, emerging sharp-tipped hairs from the follicle grow downwards or parallel to the skin and penetrate the skin (dermis or epidermis) a short distance away from the follicle, called extra-follicular penetration. Such hair-shaft invagination of the dermis or epidermis causes a foreign body reaction manifest as follicular or peri-follicular papules or pustules. In cases where the hair has been pulled before cutting, by shaving against the grain or stretching the skin, the sharp tip of cut hair may retract into the follicle and penetrate the follicular wall underneath the skin (trans-follicular penetration) to produce foreign body reaction.2 PFB has been linked to the use of various instruments for shaving, especially multi-blade razors, as well as to improper razor use in shaving techniques such as the use of blunt blades or shaving against the grain that may increase the chance of trans-follicular penetration. Other causative links are: dry shaving (not moisturizing the hair before shaving) thus producing sharp beveled tips that encourage extra-follicular penetration, infrequent shaving that allows hair growth to a length that leads to penetration, and plucking of hair by various means that leave behind hair fragments under the skin, causing inflammatory reactions.34

Therapeutic objectives for PFB management include managing ongoing inflammation and its complications and preventing the development of new lesions. Patients presenting with moderate to severe inflammations are treated with topical anti-inflammatory and antibiotic medications, which reduce inflammation by reducing contamination by the normal skin flora that may contribute to inflammation, and also prevent the development of secondary infection.2 Shea butter is the natural fat obtained by extraction from seeds of the tree, *Vitellaria paradoxa* C.F. Gaertner, family Sapotaceae, indigenous to the East, West and Central African sub-regions,5 which has been used in the preparation of cosmetics and skin care products for decades.6 It has demonstrated emollient property in eczema treatment7 and is well suited on human skin in topical products.8 *Theobroma cacao* L. or cocoa (family Sterculiaceae) is similarly an important agricultural and economic crop that grows in several tropical areas namely, West Africa, South America and Central America.9 Following the removal of cocoa beans (the main economic part of cocoa pods, used for chocolate manufacture), large quantities...
of cocoa pod husks are left as a by-product largely underexploited. However, studies have been reported on prospective pharmaceutical applications of T. cacao pod husk including its use as an excipient,11 for the production of tablets;12,13 the use of its chromatography-separated extracts as antiwrinkle agents for human skin14,15 and, more recently the methanolic and aqueous extracts of T. cacao pod husk ash (CPHA) were adjudged suitable for use as pharmaceutical ingredients through demonstrating appropriate physicochemical and antimicrobial properties.16 In view of the identified pharmaceutical use potential of CPHA extracts, therefore, the present work has been carried out to determine the extracts’ emulsifying properties, evaluate and explore the antibacterial activities of resulting emulsion products in combination with shea butter, for the development of a topical antibacterial lotion formulation useful for prospective treatment of after-shave bumps.

Materials and methods

Ethical clearance

This study was approved by the Ethics and Research Committee of Obafemi Awolowo University Teaching Hospitals Complex Ile-Ife Nigeria, and given ethical clearance certificate numbers: NHREC/27/02/2009a (national) and IRB/IEC/0004553 (international).

Collection, authentication, ashing of T. cacao (cocoa) pod husk and extraction of ash

Freshly harvested, ripe cocoa (T. cacao L.) pods were procured from cocoa farmers residing in Ile-Ife Nigeria. A specimen of the cocoa pod and leaves was prepared, authenticated, assigned the catalogue number IFE 17464 and deposited at the Ile Herbarium, Department of Botany, Obafemi Awolowo University (OAU), Ile-Ife Nigeria. Following removal of the beans, the broken cocoa pods were washed, sun-dried for 14 days, turning them over constantly to hasten the drying process. They were further dried in an hot-air oven (at 45 °C) and kneaded in a porcelain mortar with pestle to liberate shea fat from the nut pulp mass. The frothy oily mass was washed with water to remove dirt particles, scooped into a pot and boiled until neat shea oil separated, floating on top of aqueous sediment. The shea oil was collected by decantation and filtered through a Whatman No. 1 filter paper in an oven at 60 °C; and congealed as neat shea butter on cooling at the ambient temperature.

Table 1 Composition and physical stability characteristics of preliminary emulsion formulations of T. cacao pod husk ash (CPHA) extracts containing shea butter

| Formulation ID | Oil Phase: Shea butter | Aqueous phase* CPHA extract | Tween 80/ Span 80 Emulsifier** | Consistency | Pourability/ Mobility | Creaming (%)*** | Redispersibility |
|----------------|------------------------|----------------------------|-------------------------------|-------------|------------------------|----------------|----------------|
| PF 1           | 5                      | 95                        | Stable liquid formulation     | Unstable, Liquid; Very mobile | Creamed within 24 h | 60             | Readily dispersible |
| PF 2           | 10                     | 90                        | Stable liquid formulation     | Unstable, Liquid; Mobile      | Creamed within 24 h | 20             | Redispersible |
| PF 3           | 15                     | 85                        | Stable liquid formulation     | Unstable, Liquid; Mobile      | Creamed within 24 h | ≤10            | Gentle shaking required for homogenous dispersion |
| PF 4           | 20                     | 80                        | Stable liquid formulation     | Stable liquid formulation     | Mobile              | 0              | Not applicable |
| PF 5           | 25                     | 75                        | Stable liquid formulation     | Stable liquid formulation     | Mobile if gently shaken | 0              | Not applicable |

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Table 2: Composition of lotion formulations of T. cacao pod husk ash (CPHA) extract

| Components | Formulation ID/ Components’ concentration |
|------------|------------------------------------------|
|            | A         | B         | C         | D         |
| Oil Phase: | 20        | 20        | 20        | 20        |
| Shea butter (%w/w) | 2.5      | 5         | 5         | 5         |
| Aqueous phase: |          |           |           |           |
| (Dispersal medium): |          |           |           |           |
| CPHA extract (%w/v)b |          |           |           |           |
| Citrate buffer (%w/v)c | –        | 5         | 5         | 5         |
| Methyl cellulose (%w/v) | –        | –         | 2         | 3         |
| Methyl paraben (%w/v) | 0.2      | 0.2       | 0.2       | 0.2       |
| Propyl paraben (%w/v) | 0.1      | 0.1       | 0.1       | 0.1       |

Key:
- Formulation ID: Identity codes of lotion formulations
- a: Concentration parameters of the components are in parenthesis
- b: Values indicate concentration of methanolic CPHA extract in the dispersion medium (aqueous phase)
- c: The citrate buffer (pH 4.0) consisted of two ingredients: citric acid monohydrate and trisodium citrate at 1.5 and 3.5 %w/v, respectively
- Ingredient so indicated was absent in the lotion formula

Collection of volunteers’ after-shave bump swabs

Using sterile swab sticks moistened with sterile water, after-shave bump swab of the beard area of each of 15 male adult volunteers aged between 25 and 40 years was collected aseptically in accordance with the approved study protocol. All the volunteer participants in the study were graduate or undergraduate students of the Obafemi Awolowo University (OAU), Ile-Ife Nigeria. Informed consent of each participant was appropriately obtained prior to their participation. Each participant filled a questionnaire that documented their individual data, namely: age, shaving history, frequency, method and equipment, after-shave signs and symptoms. Participants having after-shave bumps at the time of sampling, who were not using and had not used any after-shave preparation for two weeks prior to the sample collection, and were not on any antibiotic or anti-inflammatory medication and did not have any visible sign of topical (facial skin) fungal infection, met the inclusion criteria for the study.

Studies on microbes isolated from after-shave bump swab

The bump swab samples from volunteers were individually plated within 1 h of collection to obtain pure microbial colonies on nutrient agar and Sabouraud dextrose agar plates and incubated at 37 °C and 25 °C, respectively, code-numbering the plates such that the resultant isolates were linkable to the code-identity of each volunteer, respectively. Colonies growing on the plates within 24 to 48 h of incubation for bacteria and within 5 days for fungi were subcultured aseptically onto fresh plates of mannitol salt agar and nutrient agar for bacterial growth and Sabouraud dextrose agar for yeast or fungal growth. All the pure isolates resulting from the subcultures were then preserved in cryopreservation medium in cryovials (Nalgene, Rochester NY, USA) stored at −4 °C,19 until needed for determination and evaluation. Each isolate was recovered from cryovial storage with a sterile inoculating loop and subcultured onto nutrient agar, mannitol salt agar (for bacteria) and on Sabouraud dextrose agar plates (for yeast/fungi) and incubated at 37 and 25 °C for 24–48 h, as appropriate for bacterial and yeast/fungal growth, to produce isolated colonies, respectively. The morphological characteristics of the colonies produced by each revived isolate on the respective media (colour, average size, margin configuration, surface elevation and consistency) were noted, and the Gram-stain reaction of each facial swab isolate was determined, using the pure cultures of Escherichia coli ATCC 25922 and Staphylococcus aureus NCTC 6571 as the Gram-negative and Gram-positive controls, respectively. Biochemical tests were also carried out for further identification of the recovered facial swab isolates (namely: catalase test, slide and tube coagulase tests, and the modified oxidase test) in order to distinguish between micrococci and staphylocci,19 following the standard methods described by Barrow and Feltham,20 using Pseudomonas aeruginosa ATCC 10145 and E. coli ATCC 25922 as the positive and negative controls, respectively.

Antibiotic susceptibility testing of microbes isolated from after-shave bump

The bacterial isolates were tested for their susceptibilities to 3 antibiotics namely: ciprofloxacin (5 µg) (Cypress Diagnostics), erythromycin (15 µg) (Liofil Chem, Italy) and tetracycline (30 µg) (Liofil Chem, Italy), in order to ascertain identities of similar-characteristic isolates by differentiating them on the basis of antibiotic susceptibilities. The standard agar diffusion method using antibiotic discs, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines,21 was used:

A few distinct colonies of each isolate or test organism growing on a nutrient agar plate in previous 24 h were transferred with a sterile loop into 5 ml of sterile water in a test tube. The suspension was thoroughly mixed with a spin mixer and adjusted to a turbidity of approximately 10° cfu/ml, using the 0.5 McFarland standard. The suspension was then evenly spread on the surface of an over-dried Mueller Hinton agar plate with a sterile cotton tipped applicator (Sterilin Ltd, Middlesex, UK). The inoculated plates were incubated at 37 °C for 20 min for acclimatization and growth of the inoculum. The antibiotic discs were then lightly but firmly pressed onto the surface of the agar, equidistant to each other using sterile forceps. The plates were refrigerated at 4 °C for 30 min to permit diffusion of the antibiotics, and afterwards incubated at 37 °C for 18 to 24 h. S. aureus NCTC 6571, Bacillus subtilis NCTC 8263, E. coli ATCC 25922, and P. aeruginosa ATCC 10145 were used as control strains. The diameters of zones of inhibition were thereafter measured in duplicate tests, and interpreted according to the CLSI manual.21

Determination of antimicrobial activities of T. cacao pod husk ash (CPHA) extract lotion formulations

Antimicrobial activities of CPHA-extract lotion formulations (A, B, C, and D; Table 2) and of a comparator product, Neo-Medrol® after-shave lotion (Pfizer Inc. USA; containing 1.75 mg/ml neomycin sulphate as active principle) as positive control were determined against bacterial isolates from the after-shave facial bumps of volunteer subjects and reference organisms namely: S. aureus NCTC 6571, B. subtilis NCTC 8263, P. aeruginosa ATCC 10145, and E. coli ATCC 25922, using the agar-well diffusion assay as follows. Twenty milliliters of melted and cooled nutrient agar plates were seeded with 0.2 ml (approx. 1×10⁸ cells per ml) of an overnight nutrient broth culture of each test organism and allowed to set. Three equidistant 7-mm diameter wells were cut into the plates with a sterile cup borer and 100 µl of each lotion sample for testing was pipetted into seven wells of the plates.
separate agar wells such that each of the control and the formulated CPHA-extract lotion samples challenged each organism in duplicate experiments. The test products were allowed one hour to diffuse into the agar, and the plates were incubated at 37 °C for 24-48 h. The inhibition zone diameters were thereafter measured.

Preservative efficacy testing

Preservative efficacy evaluation was carried out on two CPHA-extract lotion formulations (B and C; Table 2) that earlier showed superior desirable qualities, using 4 test organisms namely: 5. aureus NCTC 6571, P. aeruginosa ATCC 10145, Candida pseudotropicalis NCYC 6, and Candida albicans (an isolate obtained from the Department of Microbiology, OAU, Ile-Ife, Nigeria), according to compendium procedure. An overnight nutrient agar slope culture of the bacterial strains and an overnight Sabouraud dextrose agar slope culture of the yeast strains was washed, respectively, with 0.1 % peptone water and diluted serially with same medium to provide the test inoculums. Duplicate test lotion samples (9.9 ml each) dispensed in sterile MacCartney bottles were inoculated with 0.1 ml inoculum of one of the four challenge organisms, respectively, such that each 10.0 ml inoculated sample contained approx. 10⁶ colony forming units per milliliter (cfu/ml) of the inoculated cells, respectively. Peptone water in MacCartney bottles (0.1 %; 9.9 ml) was also inoculated with 0.1 ml of each test organism, respectively, as positive controls; while 10 ml samples of each lotion formulation dispensed in MacCartney bottles, which were not inoculated with any organism, were used in parallel experiments as negative controls. Each test sample was shaken for uniform distribution and stored at the ambient temperature (29±2°C) until periodic sampling times. At each sampling time (namely: 0, 6, 48 h; 7, 14, and 28 days), 0.1 ml of the inoculated lotion sample (or control), well mixed, was introduced into 9.9 ml of 0.1 % peptone water containing 1 % Tween 80, mixed thoroughly by vigorous shaking, and further serially diluted 1 in 100 aseptically with sterile water. 0.5 ml aliquots of the latter dilution were then plated in duplicate onto sterile nutrient agar plates for bacteria, and Sabouraud dextrose agar plates for yeasts, and incubated aerobically at 37 °C for 24 h, and 25 °C for 48 h, respectively. 0.5 ml aliquot dilutions of the positive and negative control preparations were similarly plated out at 0 h along with the challenged products. The average number of colony forming units recovered of each test organism following the respective incubation periods was determined.

Results

Stability properties of CPHA extract lotion formulations

Emulsion type, consistency and stability

Regardless of which CPHA extract and its concentration (methanolic 2.5 or 5.0 %; or aqueous 4.0 %w/v) was used for preparation, preliminary formulations of CPHA extract emulsion-lotion containing 5-25 %w/v of shea butter (PF 1-5, 9, and 10; Table 1) were pourable liquid oil-in-water (o/w) emulsions, and dispersible on creaming (hence generally stable) immediately and within 1 h after preparation. But the other formulations containing 30-40 %w/v shea butter (PF 6-8 and 11-14; Table 1) were viscous, not pourable and some of them congealed into semisolid consistency, prone to breaking when agitated. Pourable preliminary formulations containing 15-25 %w/v of shea butter and made without Tween 80® and Span 80® (non-ionic emulsifiers: PF 3-5; Table 1) exhibited little or no creaming over 24 h and were better in other stability characteristics during initial 7 days after preparation compared to the pourable formulations containing the emulsifiers (PF 9-11; Table 1). In summary, 20 and 25 %w/v shea butter concentrations used for CPHA-extract lotion formulation yielded the most desirable lotion consistency: showing no cracking, minimal creaming and dispersibility. The CPHA-extract lotion formulation A (Table 2) exhibited a strong alkaline pH value (11.55±0.13), which was lower than that of neat CPHA (methanolic extract aqueous solution (5 %w/v; pH=12.98±0.03). However, on incorporation of citrate buffer (5 %w/v) into the formulations (B, C, and D; Table 2) the pH value was neutralized to about 7.0 (Table 3), making them more suitable for topical application. Also, the presence of methyl cellulose (2 and 3 %w/v) in the lotion formulations (C and D, respectively; Table 2) increased their viscosity, resulting in considerable decrease in their creaming over time compared to formulations A and B that did not contain the viscosity imparting agent.

Table 3 Physicochemical stability properties of T. cacao pod husk ash (CPHA) extract lotion formulations

| Formulation ID/ Sampling time | A | B | C | D |
|------------------------------|---|---|---|---|
| **Phyiscochemical stability indicators** | Day 1 | Wk 1 | Wk 2 | Wk 4 | Day 1 | Wk 1 | Wk 2 | Wk 4 |
| pH | 11.9±1.0 | 11.9±0.5 | 11.9±0.7 | 12.1±0.8 | 6.7±0.7 | 6.7±0.7 | 6.8±0.8 | 7.1±0.6 |
| Viscosity (cP) | 2.5±1.0 | 3.0±2.0 | 3.5±2.0 | 2.5±2.0 | 4.5±1.0 | 6.5±1.0 | 6.5±1.0 | 5.0±1.0 |
| Creaming (%) | 0 | 40±2 | 55±3 | 62±3 | 0 | 32±2 | 35±2 | 37±3 |
| Droplet diameter (µm) | 3.2±2.6 | 5.0±3.9 | 6.9±4.8 | 8.0±5.7 | 3.2±3.6 | 4.7±2.7 | 6.6±4.0 | 7.8±3.6 |
| **Phyiscochemical stability indicators** | Day 1 | Wk 1 | Wk 2 | Wk 4 | Day 1 | Wk 1 | Wk 2 | Wk 4 |
| pH | 6.0±0.9 | 7.3±0.4 | 7.3±0.7 | 7.2±0.5 | 6.9±0.8 | 7.0±0.5 | 7.4±0.9 | 7.4±0.9 |
| Viscosity (cP) | 7.0±1.0 | 11.0±1.0 | 12.0±1.0 | 11.0±2.0 | 40±1.0 | 35±2.0 | 30±1.0 | 28±1.0 |
Table Continued...

| Formulation ID/ Sampling time | Creaming (%) | Droplet diameter (µm) |
|------------------------------|--------------|-----------------------|
|                              | 0            | < 5                   |
|                              | 5±1          | 7±1                   |
|                              | 0            | 0                     |
|                              | 0            | 0                     |
|                              | < 5          |                       |

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Key:
Formulation ID: Identity code of lotion formulation
*: pH and droplet-size data are expressed as Mean ± SEM; Viscosity data indicate Mean ± Range; Creaming data indicates the cream-phase volume ratio, CVR (Vu/Vo) converted to percentage (%) unit
0: 0% Creaming means, no creaming occurred in the lotion samples

Results of accelerated stability tests

In the B, C, and D lotion formulations, centrifugal force at 1000 or 5000 rpm lasting 30 or 60 min caused oil-phase separation of similar severity (p>0.05) (≤10 %; Figure 1B–1D) while a high proportion (55-100 %) of the emulsion phase (i.e. stable emulsified oil) was yet maintained in each; indicating that MC present at 2 or 3 %w/v in C and D compared to its absence in B formulations (Table 2) caused no significant difference. Presence of MC made some difference, however, in products C and D compared to those without it (A and B) where, in A (66-76 %) and in B (10-35 %) the aqueous-phase creaming-range was significantly higher (p<0.05) than the corresponding range of values for the MC-containing formulations (C and D; 0-10 % in each, respectively). The creaming process was reversed by shaking; hence it was interpreted as only a mild manifestation of instability compared against oil-separation that was not reversible by shaking. Compared to their original viscosity values, the B and C formulations subjected to freeze-thaw temperature cycling stress demonstrated a significant decrease (p<0.05) of their viscosities following the stressing cycle; while the mean droplet size of each formulation increased (but not significantly; p>0.05) due to the accelerated stability test. There was, however, no significant difference found in pH values of the formulations as a result of the temperature cycling stress (p>0.05) (Table 4).

Figure 1A Relative proportions of separated emulsion phases in CPHA-extract lotion A formulation following graded centrifugal forces.

Figure 1B Relative proportions of separated emulsion phases in CPHA-extract lotion B formulation following graded centrifugal forces.

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Figure 1C Relative proportions of separated emulsion phases in CPHA-extract lotion C formulation following graded centrifugal forces.

Figure 1D Relative proportions of separated emulsion phases in CPHA-extract lotion D formulation following graded centrifugal forces.

Table 4 Effect of freeze-thaw temperature cycling on stability of selected CPHA-extract lotion formulations

| Formulation Code/ Physical Stability Indicators/ Stability data* |
|---------------------------------------------------------------|
|                  | B                                      | C                                      |
| ----------------- | --------------------------------------- | --------------------------------------- |
|                  | pH                                     | Droplet diameter (µm)                  | Dynamic viscosity (cP) |
| Before freeze-    | 6.7±0.7                                 | 3.2±2.8                                | 4.5±0.5             |
| thaw cycling      |                                        |                                        |                      |
| After freeze-     | 7.1±0.4                                 | 5.8±3.3                                | 2.0±0.5             |
| thaw cycling      |                                        |                                        |                      |

Key:
*: pH and droplet-size data are expressed as Mean±SEM; Viscosity data indicate Mean ± Range

Incidence and susceptibility profile of volunteers’ after-shave bump swab microbes

Each of the 15 volunteers’ after-shave bump swabs gave microbial growth on nutrient agar and mannitol salt agar, but no growth on Sabouraud dextrose agar. Three or four bacterial isolates per volunteer were recovered from the shaving bump swab sampling process; and 51 isolates in all, which exhibited different cultural and morphological characteristics, were obtained from the 15 volunteers, namely: 30 isolates that were presumptive-positive identified as Staphylococci

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species (in that they fermented mannitol, growing on the agar medium as yellow colonies surrounded by yellow zone). Two isolates were Gram-positive bacilli, while all others were Gram-positive cocci that appeared in clusters or tetrads. The swab-sourced bacterial isolates thus represented the potential pathogens for secondary infection in the volunteers’ shaving bump cases. Fourteen (i.e. approx. 47%) of the 30 presumptive staphylococcal isolates showed sensitivity and susceptibility to CPHA-extract lotions (Table 5). Biochemical testing (catalase, coagulase and modified oxidase tests) revealed that all the 14 coccal isolates were catalase-positive (showing ability to evolve gas), 6 of which tested positive to slide coagulase test (i.e. visible clumping and agglutination) and so were confirmed as *S. aureus* with bound coagulase enzymes. Eleven (i.e. ≈37%) of the 30 presumptive staphylococcal isolates tested positive to tube coagulase test (showing clotting within 4 h), and these were identified as *S. aureus* with free coagulase enzymes.14 None of the isolates turned the deep brown colour of modified oxidase reagent to dark blue or purple colour within 10 s, indicating that they all were staphylococci, not micrococci.19,25 Concerning susceptibility to selected antibiotics, one strain (the S.14b) of the 30 presumptive staphylococcal isolates was mildly resistant to ciprofloxacin, 5 (the S.1b, S.3a, S.5d, S.10Ac, and S.15b) were resistant to tetracycline, and 5 (the S.5a, S.5b, S.5c, S.6a, and S.12b) were resistant to erythromycin; while 29, 25, and 25 of the isolates were susceptible to ciprofloxacin, tetracycline, and erythromycin, respectively (Table 5). Among the comparator organisms studied, *P. aeruginosa* was found resistant to both tetracycline and erythromycin. Overall, ciprofloxacin demonstrated the widest activity spectrum (Table 5). These results have value for possible antibiotic treatment.

### Table 5  
Susceptibility of facial-swab microbial isolates and type organisms to antibacterial activities of selected antibiotics, CPHA-extract lotion formulations and control

| Test organism/ facial-swab isolate ("S") | Ciprofloxacin (5 µg) | Tetracycline (30 µg) | Erythromycin (15 µg) | Control after-shave lotion (Neo-Medrol®) |
|----------------------------------------|----------------------|----------------------|----------------------|-----------------------------------------|
|                                        | A (12±1)             | B (12±1)             | C (12±1)             | D (12±1)                                |
| *S. aureus* NCTC 6571                  | 25.0±0.5             | 25.0±0.5             | 27.0±0.5             | 29±1                                    |
| *B. subtilis* NCTC 8263                | 23.0±0.5             | 15.0±0.5             | 23.0±0.5             | 23.5±0.5                                |
| *P. aeruginosa* ATCC 10145             | 33.0±0.5             | 0                    | 0                    | 25.5±0.5                                |
| *E. coli* ATCC 25922                   | 27.0±0.5             | 16.0±0.5             | 0                    | 29±1                                    |
| *S.1b*                                 | 29.0±0.5             | 0                    | 10.0±0.5             | 24.5±0.5                                |
| *S.1c*                                 | 28.0±0.5             | 12.0±0.5             | 23.0±0.5             | 22±1                                    |
| *S.2b*                                 | 28.0±0.5             | 23.0±0.5             | 26.0±0.5             | 24.5±0.5                                |
| *S.3a*                                 | 22.0±0.5             | 0                    | 18.0±0.5             | 29±1                                    |
| *S.3c*                                 | 25.0±0.5             | 18.0±0.5             | 25.0±0.5             | 29±1                                    |
| *S.5a*                                 | 23.0±0.5             | 19.0±0.5             | 0                    | 25.5±0.5                                |
| *S.5b*                                 | 31.0±0.5             | 22.0±0.5             | 0                    | 26.5±0.5                                |
| *S.5c*                                 | 25.0±0.5             | 19.0±0.5             | 0                    | 29.5±0.5                                |
| *S.5d*                                 | 28.0±0.5             | 0                    | 25.0±0.5             | 20±1                                    |
| *S.6a*                                 | 28.0±0.5             | 20.0±0.5             | 0                    | 29.5±0.5                                |
| *S.6b*                                 | 24.0±0.5             | 19.0±0.5             | 24.0±0.5             | 26±1                                    |
| *S.7a*                                 | 23.0±0.5             | 24.0±0.5             | 26.0±0.5             | 28±1                                    |
| *S.7b*                                 | 25.0±0.5             | 20.0±0.5             | 26.0±0.5             | 25.5±0.5                                |
| *S.8a*                                 | 28.0±0.5             | 28.0±0.5             | 26.0±0.5             | 29±1                                    |
| *S.8b*                                 | 26.0±0.5             | 23.0±0.5             | 25.0±0.5             | 30±1                                    |
| *S.8c*                                 | 27.0±0.5             | 18.0±0.5             | 21.0±0.5             | 24.5±0.5                                |
| *S.9b*                                 | 31.0±0.5             | 20.0±0.5             | 24.0±0.5             | 28±1                                    |
| *S.9c*                                 | 26.0±0.5             | 19.0±0.5             | 25.0±0.5             | 22±1                                    |
| *S.10Aa*                               | 36.0±0.5             | 28.0±0.5             | 32.0±0.5             | 26±1                                    |
| *S.10Ab*                               | 28.0±0.5             | 24.0±0.5             | 25.0±0.5             | 27.5±0.5                                

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Table Continued...

| Test organism/ facial-swab isolate ("S") | Ciprofloxacin | Tetracycline | Erythromycin | Control after-shave lotion (Neo-Medrol®) |
|-----------------------------------------|--------------|--------------|--------------|----------------------------------------|
|                                         | 5 µg         | 30 µg        | 15 µg        | A           | B           | C           | D           |
| S.10Ac                                  | 27.0±0.5     | 0            | 27.0±0.5     | 0           | 12±1        | 11±1        | 12±1        | 26.5±0.5    |
| S.11b                                   | 27.0±0.5     | 21.0±0.5     | 29.0±0.5     | 0           | 0           | 0           | 0           | 24.5±0.5    |
| S.12a                                   | 22.0±0.5     | 20.0±0.5     | 9.0±0.5      | 0           | 0           | 0           | 0           | 22±1        |
| S.12b                                   | 28.0±0.5     | 25.0±0.5     | 0            | 0           | 0           | 0           | 0           | 25±1        |
| S.12d                                   | 25.0±0.5     | 25.0±0.5     | 8.0±0.5      | 0           | 15±1        | 13±1        | 12±1        | 27±1        |
| S.13a                                   | 27.0±0.5     | 22.0±0.5     | 25.0±0.5     | 0           | 14±1        | 0           | 0           | 20±1        |
| S.13b                                   | 27.0±0.5     | 22.0±0.5     | 25.0±0.5     | 0           | 0           | 0           | 0           | 21.6±0.5    |
| S.14b                                   | 9.0±0.5      | 23.0±0.5     | 27.0±0.5     | 0           | 0           | 0           | 0           | 21±1        |
| S.15b                                   | 22.0±0.5     | 0            | 23.0±0.5     | 0           | 0           | 0           | 0           | 20±1        |
| S.15c                                   | 30.0±0.5     | 17.0±0.5     | 23.0±0.5     | 0           | 0           | 0           | 0           | 28±1        |

Key:

*: Data indicates the Mean ± Range of inhibition zone diameter measurements (mm)
0: Organism was not inhibited
S: Facial swab isolate code: The figure (1-15) of the code indicates serial number for the volunteer-subjects 1-15, while the alphabets (a, b, c, d, Aa, Ab, or Ac) indicate Isolate-identification letters
The lotion formulations: A, B, C, and D are as described in Table 2

**Antibacterial activities of CPHA extract lotion formulations**

The CPHA-extract lotion formulations B, C, and D demonstrated inhibitory activities against the reference bacterial organisms: *S. aureus* NCTC 6571 and *P. aeruginosa* ATCC 10145, while the formulations B and C also showed activities against *B. subtilis* NCTC 8263. However, formulations A and D showed no activity against *B. subtilis* NCTC 8263, and *E. coli* ATCC 25922 was not sensitive to the antibacterial activities of all the formulations (A, B, C, or D; Table 5). The CPHA-extract lotions B, C, and D also demonstrated inhibitory activities against some (less than a half) of the facial swab isolates, while formulation A showed no activity against them all. On the other hand, the comparator after-shave lotion product, Neo-Medrol® showed activities against most of the isolates (Table 5) and demonstrated significantly greater (p<0.05) antibacterial effects than those of the CPHA extract lotion formulations against the isolates and reference organisms.

**Selection of optimal formulations of CPHA-extract lotion**

From consideration of its superior physical stability (Fig. 1), antimicrobial activities (Table 5) and preferable pH values (Table 3), the CPHA-extract lotion B was selected as better than the lotion A. Similarly the lotion C which demonstrated a relatively broader scope of antibacterial activities against test organisms (Table 5), and was more readily pourable due to its lower viscosity (Table 3) and contained a lower MC proportion (Table 1) hence with better promise of cost-effectiveness in scale-up production than the lotion D, was preferably selected. The B and C lotion formulations were thus selected as the optimal formulations of CPHA-extract lotion that demonstrated more desirable qualities than the others.

**Results of preservative efficacy tests**

Results of preservative efficacy tests on the CPHA-extract lotion products B and C (Table 6) showed that the yeast cells (Candida) were more vulnerable than bacterial cells to the preservative in both formulations, in that no growths of *C. pseudotropicalis* or *C. albicans* were recovered from the tested products throughout the study. On the other hand, viable cells of *P. aeruginosa* (Pa) or *S. aureus* (Sa) were found in some of the lotion samples under testing until the 6th and 48th h (Pa in product C and Sa in product B, respectively), or until the 7th day of sampling (Sa in product C). None of the inoculated organisms was, however, recovered from the formulation samples from Day 14 forward (Table 6). And whereas all positive control experiments demonstrated growths confirming each test organism’s viability, no organisms were recovered from all the negative control preparations that were not challenged with any test organisms.
Table 6 Results of preservative efficacy tests on selected CPHA-extract lotion formulations

| Sampling Time | S. aureus | P. aeruginosa | C. pseudotropicalis | C. albicans | S. aureus | P. aeruginosa | C. pseudotropicalis | C. albicans |
|---------------|-----------|---------------|---------------------|-------------|-----------|---------------|---------------------|-------------|
| 0 h           | 4×10^4    | 3×10^4        | 0                   | 0           | 4×10^4    | 6×10^4        | 0                   | 0           |
| 6 h           | 4×10^4    | 2×10^4        | 0                   | 0           | 4×10^4    | 2×10^4        | 0                   | 0           |
| 48 h          | 4×10^4    | 0             | 0                   | 0           | 2×10^4    | 0             | 0                   | 0           |
| 7th day       | 0         | 0             | 0                   | 0           | 1×10^4    | 0             | 0                   | 0           |
| 14th day      | 0         | 0             | 0                   | 0           | 0         | 0             | 0                   | 0           |
| 28th day      | 0         | 0             | 0                   | 0           | 0         | 0             | 0                   | 0           |

Key:
0: No residual contaminant was recovered

Discussion

This study describes an investigation of soap-stabilized liquid emulsion (lotion) formulations produced by shea butter emulsification in aqueous solution of cocoa pod husk ash (CPHA) methanolic extract, having antibacterial properties and suitable for use as shaving bumps (Pseudofolliculitis barbae, PFB) treatment. It provides a valorized use of cocoa pod husk as well as an additional therapeutic option for PFB. On ashing of cocoa pod husk, its potassium and high sodium contents became available as the salt residues to react with free fatty acids in a vegetable oil or fat to produce soap that could serve as anionic surfactant in an emulsion preparation. This study has demonstrated the ability of aqueous solutions of the methanolic CPHA extract to emulsify shea butter and produce stable lotions, while the extract serves as an antibacterial agent for treatment of shaving bumps. Shaving creams, soaps and gels as well as after-shave lotions and balms are widely used by men as grooming products to obtain and maintain a clean shave and smooth face. The satisfactory physicochemical stability properties of the lotions B and C overall, selected as the most suitable for PFB treatment, are due to the lotion composition and component proportions. Shea butter is an established cosmetic ingredient that is included and well tolerated by users of all ages in a variety of skin treatment products. It serves (at 20 %w/w concentration) as the oil phase giving a viscous-liquid consistency to the oil-in-water emulsion-lotion system at the ambient temperature. It was used in the formulations also for its emollient effect, which should cushion the abrasive effect of shaving on facial skin and could also aid healing of abrasions. The enhanced viscosity of lotion C due to the presence of methyl cellulose (2 %w/v) in its formula would aid its application by preventing its run off from the application site on the skin surface. Furthermore the CPHA extract, as the continuous aqueous phase, also contains potash in solution, which offers both astringent and deodorant properties. The methanolic extract of CPHA, employed in the formulations, had previously demonstrated superior antimicrobial activities and was thus preferred to the aqueous extract, in order to ensure better antibacterial effects. Consequently, the selected formulations (B and C) demonstrated inhibitive activities against proliferation of the skin commensals, S. aureus and other organisms used in the study, which could become important for opportunistic secondary infection in PFB cases. The pH of the formulations is neutral-buffered to guarantee their compatibility with skin surface fluid and preclude irritancy. Finally, the results of preservative efficacy testing of lotions meet the pharmacopeial requirement for non-sterile topical pharmaceutical preparations. The lotions as an emulsion system should be stored in a cool environment avoiding extremes of temperature fluctuation, in order to support and maintain their consistency and stability qualities.

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Conflicts of interest
Authors declare that there is no conflict of interest.

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