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Antibacterial evaluation of *Acacia nilotica* Lam (Mimosaceae) seed extract in dermatological preparations

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ABSTRACT: The plant *Acacia nilotica* Lam (*Mimosaceae*) has received a lot of recognition because of its ethno-medicinal claims, some of which have been justified by scientific studies. Various forms of its presentation like the liquor concentrates (decoction) and powders are used traditionally, however, none of these have been standardized to assure efficacy, safety, stability and appropriate dose delivery. The intention of this present work was to develop an antibacterial dermatological dosage formulation that will serve as a remedy for the treatment of skin diseases. The powdered seeds were macerated in methanol for 48 h and the resultant extract was formulated using Aqueous cream BP in which parabens was substituted for chlorocresol as preservative, while lipophilic ointment base, shea butter, was used at concentrations of 5.0, 7.5 and 10.0 % w/w respectively. The formulations were evaluated physico-chemically and subsequently tested against selected organisms such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli, Klebsiella pneumonia, Streptococcus pyrogens and Salmonella typhi* which are commonly implicated in skin infections. Ciprofloxacin and commercially available Neomycin cream were used as reference compounds. The results showed that, all the preparations especially the ointment containing shea butter gave favourable physico-chemical characteristics and at 10.0 % w/w concentration, exhibited significant antibacterial activity ($p<0.05$) against the microorganisms tested with activity against *Staphylococcus aureus* being the highest followed by *Pseudomonas aeruginosa*> *Streptococcus pneumonia*> *Klebsiella pneumonia*= *Escherichia coli*= *Salmonella typhi.* Therefore showing potential for development as a standardized dosage form for the treatment of skin infections where the interrogated organisms are implicated.

KEYWORDS: *Acacia nilotica*; shea butter; aqueous cream; antibacterial; skin.

1. INTRODUCTION

Medicinal plants and products obtained therefrom have served as sources of relief for ailments, promoting healing and maintaining good health for as long as time and existence itself. Plant compounds like other natural products are increasingly being used as non-toxic and potent substitutes for synthetically manufactured products [1].

In recent times, the increase in multi-drug resistant microbial strains and the advent of strains with decreasing susceptibility to antibiotics have attracted the attention of the World Health Organization (WHO), Scientists and Clinicians [2]. Studies have established that, bacterial resistance and antibiotic therapy failure can be related to the overuse or misuse of these drugs; the prominent being self-medication attributed to the cost of medical consultations, inadequate diagnosis, treatment and the ability of patients to purchase antibiotics without appropriate prescriptions [3, 4, 5, 6, 7, 8]. Administration of an antibiotic without prescription or on recommendation following diagnosis is most frequently accompanied by inadequate or

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sub-therapeutic dosing and duration of treatment which can modulate the bacterial virulence. In addition, bacteria also possess the inherent ability to develop resistance to therapy. A number of effective and relatively inexpensive synthetic antibacterial drugs such as neomycin and ciprofloxacin hydrochloride have been used over the years in the region. The continued use of these drugs as seen in most of the developing countries where there is chaotic drug distribution, compounded by self-medication and drug abuse as well as irrational drug prescription, increases infection-related morbidity and mortality as a result of the drugs becoming ineffective [3, 4, 5, 6, 7, 8]. Therefore, there is the need to continue to source new drugs with fewer propensities for resistance. The use of indigenous herbal medicines is an important strategy towards the attainment of this objective. This is because it would reduce cases of non-completion of therapy for economic reasons. In addition, resistance to multicomponent preparations, as is seen in herbal medicines, develops less rapidly [9]. Although more than 80 percent of the people in both the underdeveloped and the developed countries depend on herbal medicines for their medical needs, the major problem with herbal medicines in such countries still remains their poor and sometimes unhealthy presentation. Standardization of herbal medicines therefore, should be a major interest for researchers especially in Africa. A major aspect of this standardization process is the development of suitable dosage forms for these herbal medicines [9].

The plant, *Acacia nilotica* is an evergreen, single stemmed plant of the family; mimosaceae. It is indigenous to Northern Nigeria, West Africa, North Africa, and the subcontinents of India where it grows widely in the dry lands [10]. It is also known as Gum Arabic tree, Babul, Egyptian thorn, or Prickly acacia and in Nigeria, it is known as Bagaruwa among the Hausa speaking community. It is a plant of great importance in traditional, agricultural and pastoral systems [11]. Most parts of *Acacia nilotica* plant have found useful application in traditional/folklore medicine as such it is referred to as a versatile plant. Several bioactive secondary constituents like gallic acid, isoquercitin, terpenes, phenolic glycosides, volatile essential oils, ascorbic acid, carotene, calcium, magnesium and selenium contained in the plant have been indicated in its numerous biological activities [1,13,12,14]. Traditionally the leaves, flowers, fruit pods and bark have found use in various diseases like cancer, diarrhea, dysentery, hemorrhoids, tuberculosis, leprosy, fever, bronchial infections, wounds, ulcers, diabetes and also as astringent [1,15,16,17,18,19]. In some parts of West Africa, the stem bark and gum are used to treat tumors of the eye, ear, liver and spleen in some parts of West Africa while the root and woody stem are indicated in the treatment of tuberculosis and smallpox respectively [20,21]. In other regions of the world, the fresh root extracts are taken as narcotics while the stem gum dispersion is taken as an aphrodiasic; the branches of the tree is also chewed for cleansing the mouth and cleaning the teeth [22].

Several works have investigated these folklore beliefs of *Acacia nilotica* (AN) plant and suggested diverse applications, for example, El-Tahir et al [23] investigated the ethyl acetate extract of the *Acacia nilotica* fruit pods and reported that it was highly active against *Plasmodium falciparum*. Similarly, Alli et al [24 reported strong activity of the root extract of *Acacia nilotica* against *Plasmodium falciparium*, but, methanol root extract was described as been substantially active against chloroquine sensitive strains of *Plasmodium berghei* by Jigam et al [25]. In a similar but separate report, the activity of the methanol extract of the fruit pod was found to be significant in reducing blood pressure in vivo [19]. Sanni et al [26] studied and reported the antidiarrhoeal property of the ethyl acetate extract of the pod. We have previously reported from our laboratory, the time-kill kinetics of the fruit pod extract *Acacia nilotica;* we showed that, the rate of kill was both time and concentration dependent for some of the organisms (*Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus*) implicated in wound infections [27]; the present study is an advancement over that study. Other scientists who reported antimicrobial properties of the extracts of *Acacia nilotica* include, Abeer and Sanaa [28], who used clinical isolates of gram-negative and gram-positive bacteria obtained from wound abscesses of patients at Khartoum Hospital to interrogate the ethanol and chloroform extracts of the fruit, Kossi et al [29] who investigated the effect of the hydro-ethanol fruit pod extract in healing wounds due to burns and reported accelerated healing, Manga et al [30] who reported high antimicrobial activity of the aqueous and chloroform leaf extracts of this plant against known resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Pharmaceutical semisolid formulations include ointments, gels, creams, emulsions and foams which convey drugs or active moieties intended for topical applications to membranes like the skin, buccal tissue, nasal mucosa, retina tissue, ear linings and vagina mucosa [31]. Ointments are formulations that consists of a base as the drug carrier and this influences the performance of such formulations. They have lubricating properties and are preferred as obstructing coverings over the affected skin where they prevent moisture loss and increase the duration of drug release and activity at the applied site [32]. Creams on the other hand are formulations which contain one or more active ingredient dissolved or dispersed in an oil in water (o/w) or

water in oil (w/o) emulsion. They are cosmetically and aesthetically acceptable due to their soft texture, ease of application and spread on the required site and also ease of removal from the site of application [33]. Literature survey reveal no report on the formulation and standardization of extracts of *Acacia nilotica* despite the large scientific data justifying its folkloric use and efficacy. Consequently, this study was designed to develop dermatological formulations from the methanol extract of *Acacia nilotica* seeds, evaluate its physicochemical properties and assess the antibacterial activity against some organisms commonly implicated in wounds and skin infections.

2. RESULTS AND DISCUSSION

The yield of *Acacia nilotica* seed extract (ANSE) was calculated as 7.25 % w/w. This yield may require improvement via alternative means of extraction such as using highly polar organic solvents that will be able to dissolve more secondary metabolites or continuous extraction technique such as the use of Soxhlet apparatus, in order to support scale-up formulation of this extract since the plant is abundantly available in the wild, particularly in the tropics. The composition of the seed extract creams and ointments is shown in Table 1 while the organoleptic and physical properties of the formulated creams and ointments containing ANSE (*Acacia nilotica* seed extract) are shown in Table 2.

Table 1. Composition for the preparation of *Acacia nilotica* seed extract (ANSE) creams and ointments.

F0	FP	F1	F2	F3	S0	S1	S2	S3
7.5	7.5	7.5	7.5	7.5	-	-	-	-
-	-	1.250	1.875	2.500	-	1.250	1.875	2.500
-	0.025	0.025	0.025	0.025	-	-	-	-
-	0.0125	0.0125	0.0125	0.0125	-	-	-	-
-	-	2	2	2	-	-	-	-
17.50	17.50	16.20	15.59	14.97	-	-	-	-
-	-	-	-	-	25	23.75	23.13	22.50

F0 = formulation containing aqueous cream alone without preservative.
FP = formulation containing aquepus cream with preservative.
F1, F2 and F3 = formulations containig ANSE at concentrations of 5.0, 7.5 and 10 %w/w respectively.
S0 = formulation containing She butter alone.
S1, S2 and S3 = formulations containing ANSE at concentrations of 5.0, 7.5 and 10 %w/w respectively.

Table 2. Physical properties of Acacia nilotica seed extract (ANSE) creams and ointments.

Color	White	White	Brown	Dark	Dark	Light	Light	Dark	Dark
				brown	brown	yellow	brown	brown	brown
Appearance	Smooth	Smooth	Slightly rough	Slightly rough	Slightly rough	Smooth	Smooth	Smooth	Smooth
Odor	Odorless	Odorless	Characteristic	Agreeable	Agreeable	Odorless	Earthy	Earthy	Earthy
Texture	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Gritty	Gritty	Gritty
Ease of application	+++	+++	+++	++	++	+	+	+	+
Washability	+++	+++	+++	++	++	+	+	+	+
Irritancy	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Non-irritant

+ = difficult to wash off, difficult to apply.
++ = moderately easy to wash off, moderately easy to apply.
+++ easy to wash off, easy to apply.

Organoleptic evaluation of the dried extract revealed a brown shiny powder with an earthy odor. Phytochemical studies of *A. nilotica* seed extract had showed that tannins, flavonoids, glycosides and carbohydrates are present [34]. The presence of phytoconstituents in plants create appealing opportunities for the development of modern chemotherapies against wide range of microorganisms [35, 36].

The physical characteristics of the formulated ANSE creams such as the appearance, color, odor, feel on skin, texture, ease of application and removal are displayed in Table 2. The colour of the preparations with the different bases appeared from light brown to chocolate brown, increase in color intensity was observed as the concentration of the extract increased in the formulations. Generally, the colour of all the preparations was appealing hence colorants may not be necessary when scale-up production of these formulations is required.

The creams had a smooth appearance and were uniformly mixed except for the formulation with the highest concentration of extract (10%w/w) which had a gritty appearance, while the shea butter base (ointment) had smooth appearance irrespective of extract concentration. All the cream formulations were easily applied to the skin and easily removed under a running tap unlike the ointments that were greasy and could not be removed with water because of their hydrophobic nature. Both the cream and ointment preparations were non-irritating to the skin, with a soft and smooth feel. Fragrance may be introduced into the composition of the preparations to improve the odour during large scale production for aesthetic purposes and to encourage patient compliance. Extract-vehicle compatibility was observed for 90 days.

In about 30 days of storage, all the cream formulations except the formulation containing 10 %w/w of the extract showed growth of mould. This may be as a result of failure of the parabens as preservative. The use of chlorocresol as a preservative may have been more effective as it is known to have greater preserving ability than parabens [37]. Also the water content in the composition (Table 1) is a strong factor that may support microbial contamination, as the concentration of the extract increases, the water content was decreasing which may explain why 10%w/w cream formulation with least water concentration did not support mould growth unlike those with higher concentrations of water. However, all ointment formulations remained physically stable throughout the duration of storage. Lipophilic base medicaments have been identified as being hostile for microbial contamination.

The creams (F1, F2 and F3) were found to have similar pH values (3.35 - 3.79) which is acidic (Table 3). These preparations have the tendency to cause irritation of the skin upon long usage since pH is incompatible with that of the skin.

Table 3. pH of the formulated *Acacia nilotica* seed extract (ANSE) creams and ointments.

Batch	pH
F0	6.17 ± 0.06
FP	6.32 ± 0.05
F1	3.35 ± 0.02
F2	3.79 ± 0.03
F3	3.50 ± 0.05
S0	4.25 ± 0.46
S1	5.20 ± 0.56
S2	5.49 ± 1.34
S3	7.69 ± 2.10

Therefore, it will be necessary to incorporate a buffer agent to increase the pH to acceptable level (between 4 and 6); those of F0 and FP were considerably higher (6.17 and 6.32 respectively). These are blank bases without the extract, therefore alteration in the pH could be attributed to the presence of the extract in the formulations. The pH of the ointments, S1, S2 and S3 were between 5.20-7.69. The skin's protective acid mantle is a very fine, slightly acidic film on the surface of the skin acting as a barrier to microorganisms and other potential intruders that might penetrate the skin. An increase in the pH of stratum corneum can disrupt the enzymatic activities involved in keratinization, barrier restoration and antimicrobial function. Therapeutic agents and chemicals applied to the skin are essential exogenous factors that may stabilize the skin's acid mantle. Since the pH of a healthy human skin should be slightly acidic, lying between 4 and 6, the use of topical preparations with near physiologic pH are believed to be the best in the prevention and treatment of skin abnormalities.

The viscosity (Table 4) of the ointment formulations was high and increased with increase in concentration of the extract. It implies therefore that the extract had stiffer consistency thus making the viscosity much higher. There were also significant differences ($p<0.05$) in the viscosity of the formulations and the base. Generally, ointments are formulations with high viscosity which cling to the skin as films until a force is applied before it can flow. Among semi solid formulations, ointments are more viscous than creams due to the high water content in the latter, but are less viscous than pastes which usually have a high amount of powdered materials.

The effect of shear rate on the viscosity of the ointment formulations is presented in Figure 1. As shear rate increased, the viscosity of the formulations decreased. This is therefore a shear thinning behaviour which implies that when the ointment is applied to the skin by applying rubbing force, flow will be enhanced, depending on the rate of shear.

Table 4. Physicochemical properties of ointment formulations of *Acacia nilotica* seed extract.

Formulation code	Viscosity (cP)	Density (g/mL)	Extrudability (g/sec)	Spreadability (g.cm/sec)	Occlusion factor (%)
S0	91.330 ± 6.43	0.804 ± 0.012	0.0413 ± 0.0004	0.173 ± 0.005	75
S1	298.667 ± 39.46	0.996 ± 0.003	0.0450 ± 0.0014	0.196 ± 0.004	25
S2	378.000 ± 18.33	1.016 ± 0.011	0.0475 ± 0.0007	0.115 ± 0.001	50
S3	864.667 ± 8.33	1.028 ± 0.013	0.0535 ± 0.0021	0.095 ± 0.002	75

S0 = formulation containing Shea butter alone.
S1, S2 and S3 = formulations containig ANSE at concentrations of 5.0, 7.5 and 10.0 %w/w respectively.

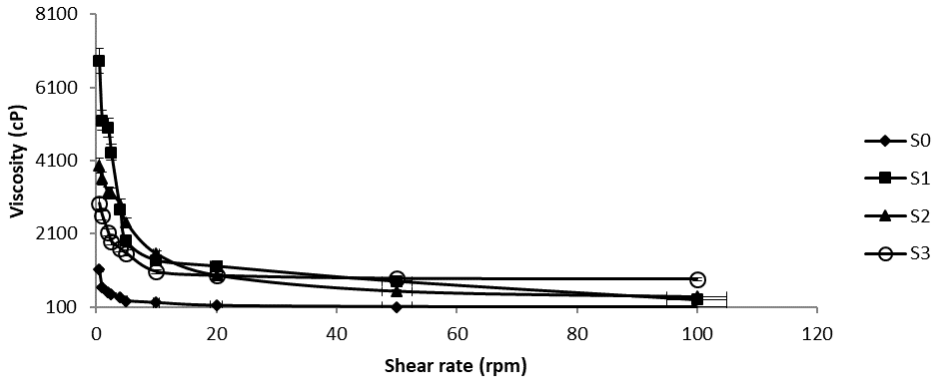

Figure 1. The effect of shear rate on the viscosity of the ointment formulations.

In addition, Figure 2 shows the rheological pattern of the ointment formulations with a plot of shear stress against shear rate and it depicts pseudoplastic rheological flow. Ointments generally cling as films until stress is applied before it begins to flow. In terms of viscosity, the ointment formulations are acceptable.

The density (Table 4) of the ointment ranged from 0.804 ± 0.012 to 1.028 ± 0.013 g/mL. The denser the formulation, the more viscous they were, hence, density is directly related to the rheology of the ointments. The ranking of the density was S0>S1>S2>S3. S3 had the highest density and it was the most viscous of all the ointment formulations while S0 had the least density and expectedly it was the least viscous.

Extrudability (Table 4) refers to the ease of removal of a semi-solid formulation like creams, ointments and gels from tubes or any material with an orifice. The extrudability of the ointment formulations ranged from 0.041-0.052 g/sec. This property can make the creams to be easily ejected from tubes and make it easier for the user. A patient that needs to apply a pharmaceutical ointment should find it user-friendly, meaning the formulation should come out easily from the tube and applied locally on the affected part of the skin. It is also important that the formulations should not run out too fast otherwise wastage will occur thus depriving the patient optimal pharmaco-economic benefit from the product. In terms of extrudability, the formulations were acceptable.

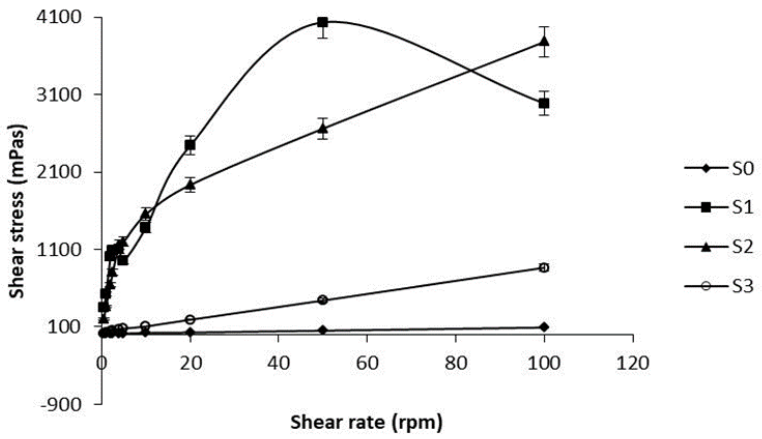

Figure 2. Shear stress versus shear rate plots for the ointment formulations.

The efficiency of topical and dermatological therapy depends on the way in which the patient spreads the formulation in even layers to administer a standard amount [38]. Spreadability denotes the extent of area to which the formulation spreads on application to the skin or affected part. Spreadability is therefore an important characteristic of these formulations and it is responsible for correct dosage delivery to the target site. Semi solid formulations should spread easily without much drag and this is usually affected by the type of base present. It should not produce friction in the rubbing process [38].

The analysis of spreadability has also been used to assess the quantitative and qualitative bioequivalence of semi-solid pharmaceutical formulations [39]. The ointment formulations spread within seconds and spreadability ranged between 0.095 ± 0.002 to 0.196 ± 0.004 g.cm/sec. This indicates that it will be easy and faster for the active constituents to be released for activity on the skin. Generally, the formulated creams in this study presented acceptable spreadability without any undue friction.

Skin occlusion by topical preparations offer diverse changes in hydration status, microbial presence, barrier permeability and lipids present in the epidermis, and may improve percutaneous absorption of chemical agents [40]. In addition, the rate of occlusion of topical formulations generally occur in the following order: occlusive film = transdermal patches > lipophilic ointments > w/o cream > oil/water cream. Occlusive vehicles are likely to increase the skin temperature by 2-3 ^{o}C resulting in increased intermolecular motion and permeability. It is therefore imperative to determine this parameter in new formulations.

The results of the occlusion factor (Table 4) for the ointment formulations was in the ranking order of S0=S1>S2>S3. The occlusion for S3 and the base, S0 could be described as moderately high. However as the extract concentration reduced, a downward shift in the occlusion properties was observed. The occlusion property of the ointment formulations is generally influenced by the lipophilicity of the extract incorporated. Methanol was used to extract *Acacia nilotica* seed and generally, unlike non-polar solvents, methanol will obtain both polar and non-polar phytoconstituents from the seed. This implies that the lipophilicity of the extract could be regarded as multidimensional.

Ointment formulation (S3) of all the preparations demonstrated inhibitory activity against the six pathogenic organisms commonly responsible for human infections. This formulation also exhibited an impressive Minimum Inhibitory Concentration (MIC) of 0.5 mg/ml, wider zones of inhibition, with the highest value being 20.0±0.5 mm and broad spectrum of activity as it inhibited both Gram positive and negative organisms (Table 5). This result is consistent with previous reports from our laboratory [27]; we had reported earlier that, in vitro time-kill kinetics antibacterial study of *Acacia nilotica* was assessed against *Pseudomonas aeruginosa, Escherichia coli and Staphylococcus aureus* determined by plate count technique and analyzed by percentage and log reduction. All test organisms were susceptible to the aqueous methanol extract. The minimum inhibitory concentration ranged between 0.5 and 1 mg, while minimum bactericidal concentration ranged between ≥1 and ≥2 mg/ml. The next active sample was reference formulation (Neomycin cream) that inhibited 3 Gram negative organisms (*E.coli, K. pneumonia and P. aeruginosa*) but the activity was not as high as that of test formulation S3 (Table 5), it is exciting that antibacterial activity of S3 compares favorably with that of ciprofloxacin and this observation corroborates natural product researchers opinion that, resistance to multicomponent preparations, as is seen in herbal medicines, develops less rapidly [9]. The formulated creams and ointments were also tested against clinical isolate of *Salmonella typhi*, and it

was only susceptible to S3 confirming the potent range effect of S3. The inhibition of the growth of the organisms observed in our study is probably as a result of disruption in the architecture of the microorganism's cell wall, leading to changes in membrane penetrability, and consequent cell destruction [41, 42].

Table 5. Inhibition zones (mm) of the formulated creams and ointments on selected microorganisms.

Samples/ Concentration (mg/mL)	*E. coli*	*K. pneumonia*	*S. aureus*	*St. pyrogens*	*P. aeruginosa*	*S. typhi*
F0 (2 mg/mL)	NA	NA	NA	NA	NA	NT
(1 mg/mL)	NA	NA	NA	NA	NA	NT
FP (2 mg/mL)	NA	NA	NA	NA	NA	NA
(1 mg/mL)	NA	NA	NA	NA	NA	NA
F1 (2 mg/mL)	NA	NA	NA	NA	NA	NA
(1 mg/mL)	NA	NA	NA	NA	NA	NA
F2 (2 mg/mL)	NA	NA	NA	NA	NA	NA
(1 mg/mL)	NA	NA	NA	NA	NA	NA
F3 (2 mg/mL)	NA	NA	NA	NA	NA	NT
(1 mg/mL)	NA	NA	NA	NA	NA	NT
S0 (2 mg/mL)	NA	NA	NA	NA	NA	NT
(1 mg/mL)	NA	NA	NA	NA	NA	NT
S1 (2 mg/mL)	NA	NA	NA	NA	NA	NT
(1 mg/mL)	NA	NA	NA	NA	NA	NT
S2 (2 mg/mL)	NA	NA	NA	NA	NA	NA
(1 mg/mL)	NA	NA	NA	NA	NA	NA
S3 (2 mg/mL)	19.0±0.3	19.0±0.3	20.0±0.5	18.5±0.4	19.5±0.5	19.0±0.3
(1 mg/mL)	18.6±0.4	18.5±0.4	17.0±0.3	17.5±0.3	17.0±0.3	8.5±0.4
(0.5 mg/mL)	16.5±0.4	17.0±0.5	8.5±0.3	16.0±0.4	16.5±0.3	NA
Ciprofloxacin (10 mg/mL)	30.0±0.4	30.0±0.3	30.0±0.3	30.0±0.5	30.0±0.3	30.0±0.3
Neomycin cream (2 mg/mL)	11.0±0.3	9.0±0.4	NA	NA	10.0	NT
(1 mg/mL)	10±0.4	9.0±0.3	NA	NA	8.0	NT

NA = No activity
NAT = Not tested
F0 = formulation containing aqueous cream alone without preservative.
FP = formulation containing aqueous cream with preservative.
F1, F2 and F3 = formulations containing ANSE at concentrations of 5.0, 7.5 and 10 %w/w respectively.
S0 = formulation containing She butter alone.
S1, S2 and S3 = formulations containing ANSE at concentrations of 5.0, 7.5 and 10 %w/w respectively.

It could also be attributed to penetration of phytochemicals into bacteria cells thus stimulating coagulation of cell contents. Tannins which are present in ANSE have been confirmed to have antimicrobial effect.

4. CONCLUSION

The ointment formulation at 10 %w/w concentration showed potentials for use in the treatment of wound and skin infections. It could therefore be developed for commercial use.

5. MATERIALS AND METHODS

5.1. Materials

Fruit pods of *Acacia nilotica* plant, liquid paraffin, emulsifying wax (Fisher Chemicals, USA), methanol (Loba Chemie, India) white soft paraffin (Fisher Chemicals, USA), Shea butter purchased from a metropolitan market in Abuja, all other chemicals used were of Analar grade.

5.2. Method

5.2.1. Collection of materials

The fruit pods of *Acacia nilotica* were obtained from the botanical garden of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria and was identified, assigned voucher no NIPRD/H/7008 and kept in the Institute's herbarium. The dried seeds were removed from the pods, pulverized and sieved using the 600 µm mesh size to obtain uniform particle sizes. The sieved material was packaged in an air-tight container and stored until further use.

5.2.2. Preparation of dried seed extracts

The dried pulverized and sieved *Acacia nilotica* seeds (293 g) was macerated in absolute methanol (1:8) at room temperature for 48 h. The supernatant was decanted and concentrated over a water bath (Karl Kobb, Derieich West Germany) at 40℃. The weight of the resulting dried product (ANSE) was determined, the product was packaged in an air-tight container and kept in a desiccator.

5.2.3. Preparation of cream formulations

Emulsifying ointment (100 g) was prepared using the official formula [43]; white soft paraffin and emulsifying wax were weighed into a beaker and allowed to melt over a water bath at 70℃ then liquid paraffin was incorporated and the mixture stirred together. This was used as the vehicle for the formulation of the creams. The creams containing the extract (ANSE) were then prepared using the composition of ingredients stated in Table 1. Appropriate quantities of the prepared emulsifying ointment was heated in a beaker over the water bath at 70℃, this served as the oily phase. The aqueous phase on the other hand was prepared by weighing the required quantity of water into another beaker and heating to 75℃ on the water bath. The preservatives (methyl paraben and propyl paraben) were also placed into the water. The oily portion was then added to the aqueous phase while stirring until completely homogenized and cooled. This water miscible base was blended together, in aliquots, with the required quantity of ANSE already in the mortar using a pestle, tween 20 was also incorporated (where necessary). The contents of the mortar were triturated together until a creamy product was obtained. The creams so prepared were packaged in appropriate containers and kept for further analysis.

5.2.4. Preparation of ointment formulations

Shea butter was used as the ointment base. Formulations containing the base and *Acacia nilotica* seed extract (ANSE) were prepared using the compositions in Table 1. Appropriate quantities of Shea butter was weighed and melted in a beaker over the water bath. The melted base was integrated in aliquots into the appropriate amount of ANSE already in the mortar using a pestle until a completely homogenous mixture was obtained. The prepared ointment was then poured into ointment jars and stored for further analysis.

5.3. Evaluation of formulated ANSE creams and ointments

5.3.1. Physical evaluation

The appearance, color, odor, texture, homogeneity, ease of application and removal, irritancy test and phase separation of the different formulated creams and ointments were evaluated.

5.3.2. Chemical evaluation

Determination of pH

The pH of the formulated undiluted creams and ointments were measured using the digital pH meter (Denver pH meter). Values for three determinations were obtained and the average calculated.

Evaluation of extract-base compatibility

The occurrence of any physical incompatibility between the extract and base in the formulated creams and ointments was visually assessed and recorded.

Evaluation of formulation stability

The prepared creams and ointments were stored at room temperature for 90 days and visually assessed for presence/absence of growth.

Determination of viscosity of ointment formulations

The Brookfield viscometer (VT 181, Karlsruhe, Germany) was used to determine the viscosity of the ointment formulations at 29.40 ± 2.5 0C. Spindle 7 was used and ten different shear rates (0.5 to 100 rpm) were applied. The shear stress was determined by calculation and appropriate plots provided.

Determination of ointment density

Syringe (2 mL) was weighed on the balance, filled with ointment sample and re-weighed. The weight of the cream was determined by subtracting the weight of the filled syringe from that of the unfilled syringe and the density was calculated in g/mL. The procedure was repeated for other ointment samples.

Determination of ointment extrudability

An empty syringe (2 mL) was weighed, and the syringe was filled with the ointment sample and then reweighed. The rate of extrusion was standardized (1press/sec) and the ointment was released. The time it takes the cream to be extruded from the syringe completely was recorded using stop watch. The procedure was repeated for other cream samples. Extrudability was then calculated in g/sec. The procedure was repeated for other ointment samples.

Determination of ointment spreadability

Ointment was weighed (500 mg) unto a slide and another slide used to cover it. A weight of 200 g was placed on top of the cover slide and the ointment was allowed to spread maximally for five minutes. Without removing the cover slide, the extent of ointment spread was measured in four different directions and the average obtained in cm. The time used to separate the upper slide from the lower was also obtained using a stop watch. Spreadability was calculated in g.cm/sec. The procedure was repeated for the remaining ointment samples.

Determination of occlusion properties

Each ointment formulation was carefully weighed (200 mg) on a filter paper and evenly spread out. This was then placed on a clean sample bottle having 2.3 diameter surface. The average perimeter of the filter paper was 22.9 cm. Distilled water (20 mL) was carefully transferred into each bottle and the filter paper containing the spread cream was placed on top to cover the entire orifice of the bottle. The water loss was determined at 24-144 h; controls with ordinary filter paper i.e. positive control (CP) and without any filter paper i.e. negative control (CN) were set up as well. Readings were taken every 24 h. The occlusion factor (F) was calculated using the equation below:

$$F = (A-B/A) X 100 \quad \text{(Eq. 1)}$$

where, B is the water loss from the test formulations; A is the water loss of the filter without a sample (blank reference). An occlusion factor of zero indicated no occlusive effect compared with the reference, and 100 was the maximum occlusion factor [44].

5.3.3. Microbiological assay of prepared ANSE creams and ointments

Overnight broth cultures of the test organisms viz; *Staphylococcus aureus* ATCC, *Pseudomonas aeruginosa* ATCC, *Escherichia coli* ATCC and clinical isolates of *Klebsiella pneumonia*, *Streptococcus pyrogens* and *Salmonella typhi* from Diagnostic Laboratory of National Institute for Pharmaceutical Research and Development were diluted to 107 cfu/mL using a UV-vis spectrophotometer [45] and plated on Nutrient agar. Two to three colonies of 20 h growth on Mueller Hinton agar of the organisms to be studied were suspended in 50 mL pre-warmed (37℃) Mueller Hinton broth. This was incubated overnight at 37℃ diluted 1/2500 using the medium and maintained in water bath while being agitated (50 rpm). The optical density of the culture was observed at 450 nm until an absorbance of 0.1 equivalent 2.5-3.0 x 107 cfu/mL for *E.coli* and *P.aeruginosa* and 1.8-2.0 x 107 for *S. aureus* was reached. Molten Mueller Hinton agar (25 mL) maintained in a water bath at 50℃ was inoculated with 100 µL standardized organism, allowed to gel in sterile Petri dish. Equidistant grooves of 5 mm diameter was bored, sealed with a drop of agar and filled with 200 µL (2, 1 mg respectively) of cream solution prepared by dissolving 1 g in 10 mL of 50% aqueous tween 80, further diluted by 1:10 in sterile broth as experimental solution. The plates were allowed to stand for 2 h and incubated at 37℃ for 18-24 h. Post incubation, the zones of inhibition were determined by measuring the zones where there was no growth.

This process was performed twice to obtain data in duplicates. Control plate of ciprofloxacin 10 µg/ml (HIMEDIA Laboratories Pvt. Ltd, India).

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