Formulation of antimicrobial cream from the leaves and bark of *Lannea welwitschii* for wound healing.

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ABSTRACT

Background: Recently there has been a remarkable surge of interest in natural products and their applications in the cosmetic industry. Topical delivery of antimicrobials from natural sources like *Lannea welwitschii* is one of the approaches used to treat wounds due to its diverse clinical and pharmacological potentials.

Objectives: The aim of this study was to develop an oil-in-water cream formulation of the methanol extract of the leaves (LF) and bark (BF) of *Lannea welwitschii* (Hiern) Engl. (Fam. Anacardiaceae) for topical delivery in the treatment of wound infections.

Methods: The antimicrobial activity of the extract was investigated. The cream was formulated using fusion method. The oil-phase ingredients were slowly transferred into the aqueous phase at a temperature of 70 °C including the extracts 1%w/w with constant stirring. The formulations upon cooling were evaluated for pH, skin irritancy, viscosity, colour and odour. The stability of the cream over 30 days was investigated so also the wound healing properties of the cream was studied in 12 albino rats using the excision wound healing model.

Results: The minimum inhibitory concentration for both the leaf and bark extract was 4mg /ml.
The extracts were most active against *Proteus mirabilis* with the leaf extract being more active than the bark extract. The extracts also had activity against *Bacillus subtilis* and *Pseudomonas aeruginosa,* with mean inhibition zone diameter ranging from 10-26mm. There was no antifungal activity observed. The four resulting cream formulations were homogenous, highly viscous, non-greasy, slightly acidic and non-irritant. The cream formulations from the leaf and bark extract were green and red respectively. There was no significant change in the evaluation parameters at 25 °C over a period of four weeks. The formulations appeared stable over the period. Statistical analysis showed that after a twenty-one day treatment on an excised wound, the two formulations showed comparable wound-healing with the standard (Cicatrin®). LF2 showed a significantly higher rate of wound closure ($P < 0.05$) than Bf2.

Conclusion: A standardized cream formulation of the extract from the plant *Lannea welwitschii* for wound healing was developed. The cream was found to be aesthetically acceptable, stable, therapeutically effective, and, hence has promising wound healing potentials in the health care industry.

Keywords: *Lannea welwitschii*, methanol extract, wound-healing, antimicrobial cream formulation.

Formulation de la crème antimicrobienne à partir des feuilles et de l'écorce de Lannea welwitschii pour la cicatrisation des plaies

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RESUME

Contexte: Récemment, l'intérêt dans les produits naturels et leurs applications dans l'industrie cosmétique ont connu un essor remarquable. L'administration topique d'antimicrobiens à partir de sources naturelles comme *Lannea welwitschii* est l'une des approches utilisées pour traiter les plaies en raison de ses divers potentiels cliniques et pharmacologiques.

Objectifs: Le but de cette étude était de développer une formulation de crème d'huile-dans-l'eau de l'extrait de méthanol des feuilles (LF) et de l'écorce (BF) de *Lannea welwitschii* (Hiern) Engl. (Fam. Anacardiaceae) pour l'administration topique dans le traitement des infections de plaie.

Méthodes: L'activité antimicrobienne de l'extrait a été étudiée. La crème a été formulée en utilisant une méthode de fusion. Les ingrédients de la phase huileuse ont été lentement transférés dans la phase aqueuse à une température de 70° C, y compris les extraits à 1% en poids/poids sous agitation constante. Les formulations au refroidissement ont été évaluées pour le pH, l'irritation cutanée, la viscosité, la couleur et l'odeur. La stabilité de la crème sur 30 jours a été étudiée de même que les propriétés cicatrisantes de la crème ont été étudiées chez 12 rats albinos en utilisant le modèle de guérison des plaies par excision.

Résultats: La concentration inhibitrice minimale pour l'extrait de feuilles et d'écorce était de 4 mg/ml. Les extraits étaient plus actifs contre *Proteus mirabilis* avec l'extrait de feuille plus actif que l'extrait d'écorce. Les extraits ont également eu une activité contre *Bacillus subtilis* et *Pseudomonas aeruginosa*, avec un diamètre moyen d'inhibition compris entre 10 et 26 mm. Aucune activité antifongique n'a été observée. Les quatre formulations de crème résultantes étaient homogènes, très visqueuses, non graisseuses, légèrement acides et non irritantes. Les formulations de crème provenant de l'extrait de feuille et d'écorce étaient respectivement de couleur verte et rouge. Il n'y a pas eu de changement significatif dans les paramètres d'évaluation à 25 ° C sur une période de quatre semaines. Les formulations sont apparues stables au cours de la période. L'analyse statistique a montré qu'après un traitement de vingt et un jours sur une plaie excisée, les deux formulations présentaient une cicatrisation comparable avec le standard (Cicatrin®). LF2 a montré un taux de fermeture de la plaie beaucoup plus élevé ($P < 0,05$) que BF2.

Conclusion: Une formulation normalisée de crème de l'extrait de la plante *Lannea welwitschii* pour la guérison des plaies a été développée. La crème a été jugée esthétiquement acceptable, stable, efficace sur le plan thérapeutique et, par conséquent, a des potentiels prometteurs de guérison des plaies dans l'industrie des soins de santé.
Mots-clés: *Lannea welwitschii*, extrait de méthanol, cicatrisation, formulation de crème antimicrobienne.

INTRODUCTION

To date, the obvious outcome measure for evaluating interventions in wound healing has been complete healing.[1] Appropriate wound care and treatment promotes healing and reduces the risk of infections, which causes delayed healing of wounds. The incidence of microbial resistance of microorganisms to antibiotics and chemical agents, the therapeutic failure of orthodox medicine for treatment of certain wound infections, and the incurability of certain ailments with orthodox medicines have driven a renewed interest in researchers to explore natural products for possible solutions.[2] *Lannea welwitschii* (Hiern) Engl. (Fam. Anacardiaceae) is a plant that grows in the tropics. It is locally known as Kuntunkuri in Ghana[3], Yorubas in the south west of Nigeria call it Ekika. The trunk bark has been reported to be used for the treatment of diarrhoea, anaemia and haemorrhoids.[2] Decoction of the leaves has been used for the treatment of diarrhea, dysentery, swellings, gout, gingivitis, topical infections, and wounds.[3] The roots are used as an antidote for food poisoning, nasopharyngeal infections and as emetics. The stem bark has also been found to contain glycosides, tannins, and saponins and used in wound healing.[3] The bark has been found to possess antidiarrheal property and two cytotoxic compounds; namely, lanneaquinol and 2-(R)-hydroxy lanneaquinol have been isolated from the plant.[4]

Regardless of the vast activity that this plant demonstrates, its use is limited to the rural dwellers due to the local and unstandardized form in which it is administered. It is therefore imperative that the crude extract be formulated into an acceptable topical dosage form and evaluated for its wound healing potential. The aim of this study is to develop and evaluate the *in vivo* wound healing properties of an oil-in-water cream formulation of the methanol extract of the leaves and bark of *Lannea welwitschii* .

MATERIALS AND METHODS

The leaves and bark of *Lannea welwitschii;* Methanol (LC-MS grade) and water (HPLC grade) were purchased from Fisher Scientific (Fair Lawn, NJ, USA), Mueller Hinton agar and Sabouraud Dextrose Agar were purchased from Becton Dickinson, (Cockeysville, Md. U.S.A.), Levofloxacin, Triethanolamine, Tween 80, Propylene glycol and Stearic acid were purchased from Sigma Aldrich (St. Louis, MO, USA). All other solvents and reagents used were of analytical and pharmaceutical grade.

Collection of plant materials

The leaves and bark of *Lannea welwitschii* were collected in February, 2015 from Ikire, Osun state, Nigeria and identified at the Herbarium of the Department of Botany, Faculty of Biological Science, University of Lagos, Lagos State, Nigeria by Mr Odewo; a voucher specimen (LUH 5718) was issued and deposited in the same herbarium.

Extraction

The leaves and bark of *Lannea welwitschii* were dried in the oven at a temperature of 60°C for 7-10 days, after which they were ground separately to a fine powder using an electronic grinder lab mill, (Chelmsford, England). The resultant weight of the powdered leaves and bark were 450g and 750g respectively. The extraction technique employed to obtain a crude extract was maceration that involves leaving the pulverized plant to soak in a suitable solvent in a closed container. Optimal extraction from the powder portions of the leaf and bark using methanol was done as reported by previous reseachers.[2]

Phytochemical screening

Phytochemical screening[2] carried out on the leaves and bark of *Lannea welwitschii* was done to confirm the presence of free and bound anthraquinones, cardiac glycosides, tannins, alkaloids, cyanogenic glycosides, flavonoids, saponins and reducing sugars.

Microbiological evaluation

Multi-drug resistant wound isolates; bacteria including *Staphylococcus aureus, Bacillus subtilis, Propioni bacterium, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa,* and fungi including *Trichophyton mentagrophyte, Candida albicans, Penicillium spp., Saccaromyces cerevisiae* obtained from the Lagos University Teaching Hospital (LUTH), Lagos, Nigeria were used for the study. The antibacterial activity of the extract was determined using the agar well diffusion technique[3] with Mueller Hinton agar as the culture medium. Using a 1ml needle and syringe, 0.2ml of different concentrations of the leaf extract, 10 – 30 mg/ml were dispensed into three of the wells while the 4th well had propylene glycol serving as a negative control. The positive control petri dishes had the four wells containing 0.2ml of four concentrations of Cicatrin®. The plates were incubated in the lid up position at 37°C for 24hrs. Inhibition zone diameter (IZD) was measured and recorded to the nearest whole diameter. Spores of *Trichophyton mentagrophyte* and

Candida albicans calibrated in 1% Tween 80 were sub-cultured on sabouraud dextrose agar and incubated at room temperature for one week. In the antimicrobial analysis, Levofloxacin and Clotrimazole were used as reference antibacterial and antifungal compounds respectively.

Minimum Inhibitory Concentration (MIC) of the methanolic extracts of the leaves and bark of *Lannea welwitschii* were determined by diluting the extracts to various concentrations (0.25 – 128 mg/ml).

Formulation of the creams

The leaf and bark extract of *Lannea welwitschii* were used to obtain 20 g cream formulations via fusion method with constituents as shown in Table 1. The oil phase ingredients, beeswax, cetyl alcohol and stearic acid were weighed into a beaker and transferred to a water bath (Surgifriend Medicals, Uniscope SM801A, England) until they all melted. The aqueous phase however, consisting of water and triethanolamine was transferred into a larger beaker and heated in the water bath for 5mins after which glycerin and the dry extract was added to the aqueous mix. Upon solubilisation and reaching a temperature of 85 °C, the aqueous phase was removed from the water-bath and the oily phase was slowly poured into it stirring continuously until a smooth, uniform cream was obtained. The resulting cream was packaged into a plastic cream jar, labeled LF1 and BF1 for the leaf and the bark extracts respectively and stored until further tests. The same procedure was carried out on second set of formulations but Tween 80, Propylene glycol and Arachis oil was included while Beeswax was excluded (Table 1). The formulations were labelled LF2 and BF2 for the leaf and bark extracts respectively and stored until further tests.

Table 1: Details of constituents in *Lannea welwitschii* cream formulation

	AQUEOUS PHASE				OILY PHASE					
Ingredients (%w/w)	Plant extract	Water	Triethanolamine	Glycerin	Tween 80	Propylene glycol	Arachis oil	Cetyl alcohol	Stearic acid	Beeswax
LF1/BF1	1.0	70.0	2.0	5.0	-	-	-	5.0	10.0	7.0
LF2/BF2	1.0	70.0	2.0	5.0	2.0	5.0	5.0	5.0	10.0	-

***In-vitro* assessment of the formulated creams**

The prepared cream formulations with designated codes LF1, BF1, LF2 and BF2 were evaluated for the following parameters. All measurements were done in triplicates.

Homogeneity: Observing the appearance of the cream by visual inspection as well as the feel upon touch tested the homogeneity of the cream.

Evaluation of cream pH: The pH meter was calibrated using standard buffer solution. About 0.5 g of the cream was weighed and dissolved in 50.0 ml of distilled water and its pH was measured.

Irritancy- Two albino rats had a portion of the hair on their dorsal region shaved off to expose the epidermal layer of the skin. The formulated creams were applied and irritancy in terms of erythema and edema was investigated.

Rheological study

The viscosity of the cream formulations stored at room temperature (25 °C) and at an elevated temperature of 40 °C was determined using a Brookfield DV-II + viscometer with LV-4 spindle (Brookfield Engineering, U.S.A.) at a speed of 20 rpm[5]. The viscosity of the cream was determined at the time of preparation and on a five-day interval basis for a period of 30days.

Spreading coefficient

The spreading ability expressed as the time it takes in seconds for two slides to slip off from the cream placed in between them under certain loads was done as described by previous authors.[5] Two sets of glass slides of standard dimension were used for each cream formulation. The cream formulation was placed over one of the slides; the other slide was placed on the top of the cream, such that the cream was sandwiched between the two slides in an area occupied by a distance of 6.0cm along the slide. 100gm weight was placed upon the upper slide so that the cream between the two slides will be pressed uniformly to form a thin layer. The weight was removed and the excess of cream scrapped off. The two slides in position were fixed to a stand without any interference and in such a way that only the upper slide slipped off freely by the weight tied to it. A 20gm weight was tied to the upper slide carefully. The time taken for the upper slide to travel a distance of

6.0cm and separate away from the lower slide under the influence of the weight was noted. The spreadability of the cream was determined at the time of preparation on all the cream formulations. Spreading coefficient was calculated using the equation III below:

$S = M \times L / T$**.....**Equation I

Where, S is Spreading coefficient**,** M = Weight tied to the upper slide (20gm), L = Length of the glass (6cm) and T= time taken in seconds.

Evaluation of the wound healing property of *Lannea welwitschii* cream formulations

Study Animals

Twelve (12) male albino rats with their resultant weights between 121–200g were selected for evaluation of wound healing activity. The rats were used after acclimatization under controlled conditions of temperature of 25 ± 2°C, humidity of 50 ± 5% and 12 hours of light and dark cycles, respectively for 7 days. The animals were housed individually in polypropylene cages and had free access to sterile food (animal chow) and water. All experiments were performed in accordance with the recommendations and policies of the Ethics Committee of the College of Medicine, University of Lagos guidelines for the care and use of laboratory animals.

Study design

The animals were randomly allocated into four (4) groups with three (3) animals in each group. Group I received no treatment, allowing for normal wound healing process (Control); Group II received the standard drug Cicatrin® which consists of Neomycin and Bacitracin; Group III received the formulated cream LF2 containing the leaf extract, Group IV received the formulated cream BF2 containing the bark extract.

Excision wound model

The excision wound model was used for studying wound healing activity in the albino rats as reported by previous authors with some modifications.[6] Animals were anesthetized prior to and during creation of the wounds with 1ml of intravenous ketamine hydrochloride (10 mg/kg body weight). The dorsal furs of the animals were shaved and the area of the skin where the wound was to be created was circularly marked with a diameter of about 3cm. Ethanol (70%) was used as antiseptic for the shaved region before excising the wound with a pair of toothed forceps, scalpel and pointed scissors. The wound was left undressed to the open environment and no local or systemic anti-microbial agents were used. Formulated cream LF2/BF2, was applied on the wound once daily for 21 days starting from the day of excision. The observation of percentage wound closure/contraction was made and calculated at intervals up to twenty first post wounding day using Equation II below, tracing and measuring the wound margin/diameter on a tracing paper[6] in order to determine the wound contraction rate.

Wound contraction (%) = $[(WD_0 - WD_t)/WD_0]$ 100……. Equation II

Where WD_0 is the wound diameter (cm) on day zero, the wound diameter (cm) on day t WD_t.

Wound area: A = x r……..Equation III

Where, A = wound area (cm^2), = 3.142, r = radius (cm)

Statistical analysis

Results were expressed as mean ± standard deviation (SD) of three determinations. Statistical comparison were made by using one-way Analysis of variance (ANOVA), followed by Student and Dunnet multiple 't' comparison test using Graph Pad Prism software (GraphPad software Inc., Version 4.0.0.255), results were considered statistically significant when p-values were < 0.05.

RESULTS

Organoleptic properties of the extract

The percent yield of extracts obtained from the dried leaves and bark of *Lannea welwitschii* was found to be 7.5%w/w and 5.5%w/w respectively. The leaf extract had an alcoholic odour and was green in colour, difficult to scoop and slightly sticky. The bark extract was however reddish-brown in colour with no odour; very fine powder, easy to scoop and han

Table 2: Phytochemical screening of the methanol extracts of the leaves and bark of *Lannea welwitschii*

Phytochemical analysis	Leaf extract	Bark extract
Alkaloids	–	–
Anthraquinones:		
a) Free	–	–
b) Bound	–	–
Cardiac glycosides:		
a) steroidal ring	–	–
b) steroidal nucleus		
c) Cardenolides	–	–
	+++	+++
Flavonoids	+++	–
Saponins	+++	+++
Sugars:		
a) Reducing sugars	–	+++
b) Barfoed's test	+++	+++
c) Molisch's test	+++	+++
Tannins:		
a) Hydrolysable	+++	–
b) Proanthocyanidins	+++	+++
c) Phlobatannins	–	+++
Terpenoids	–	–

+ = positive - = negative

Microbiological evaluation

Microbiological evaluation carried out on the leaf and bark extracts of *Lannea welwitschii* showed highest activity against *Proteus mirabilis* with the leaf extract been more active than the bark extract. Other organisms that the extracts were active against are *Bacillus subtilis,* and *Pseudomonas aeruginosa* with mean inhibition zone diameter ranging from 10-26mm. There was no antifungal activity observed, the MIC for both leaf and bark extract obtained was 4mg/ml.

Evaluation of the formulated creams

The resulting cream formulations from the extract of the leaf and bark of *Lannea welwitschii* were smooth, homogenous, non-greasy and easily washed off the palms of the formulator with water. The leaf formulations (LF1 & LF2) were green while the bark formulations (BF1 & BF2) were red in colour. On application to the rat skin, the cream formulations were emollient; none of them produced irritation, edema or erythema. The results of the other parameters by which the cream formulations were assessed areas shown in Table 3.

Table 3: Physicochemical properties of *Lannea welwitschii* cream formulations

Formulation code	pH	Viscosity (cP)	Spreadability (g/secs)
LF1	6.6±0.01	65000±1250.33	21.85±0.01
BF1	6.5±0.00	65300±1220.00	21.81±0.03
LF2	6.2±0.00	63000±1240.01	24.00±0.02
BF2	6.3±0.01	62800±1237.22	23.95±0.01

Effect of time and temperature on formulated *Lannea welwitschii* creams.

There was no growth of organisms, no change in the colour and appearance of the formulated creams upon exposure to temperature as high as 40 °C over 30 days. The viscosity and pH of the cream formulations upon storage at 40 °C, decreased over 30 days (Figure 1 & 2). However, upon storage at 25 °C for 30 days, the pH and viscosity increased over time with the four cream formulations but not significantly (Figure 3 & 4). The LF2 and BF2 formulations gave more acceptable and consistent results judging from the evaluated parameters than the LF1 and BF1 cream formulations hence they became the choice formulation upon which the wound healing assessment was carried out.

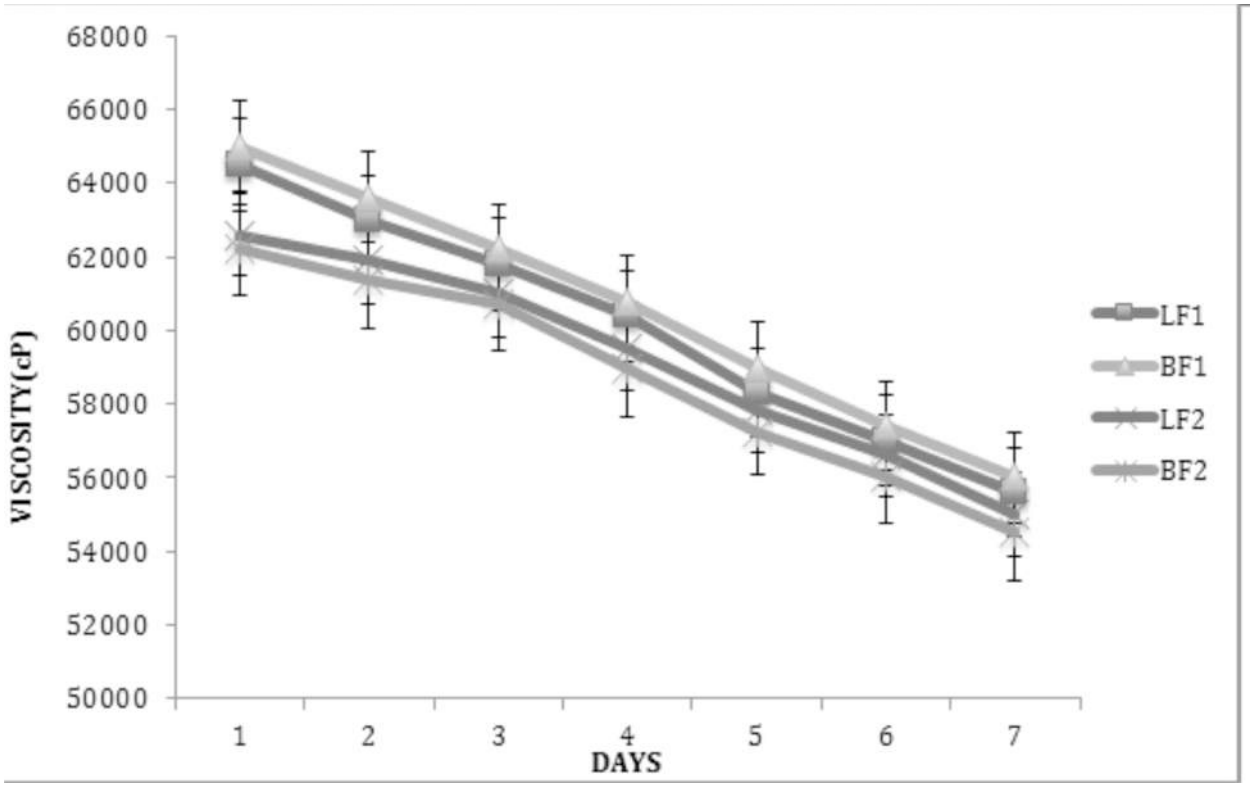

Figure 1: Effect of time on the viscosity of *L. welwitschii* cream formulations stored at 40 °C.

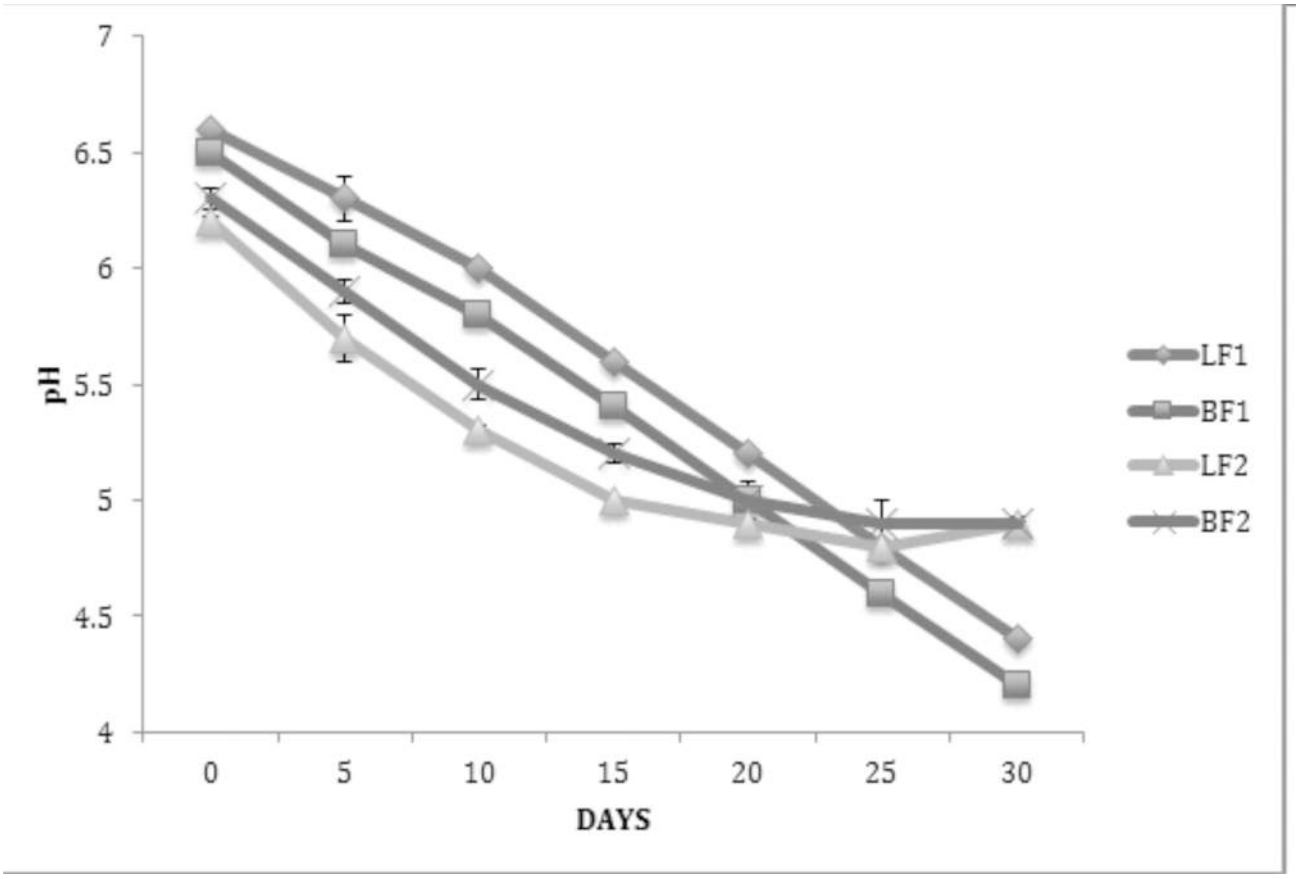

Figure 2: Effect of time on the pH of *L. welwitschii* cream formulations stored at 40 °C

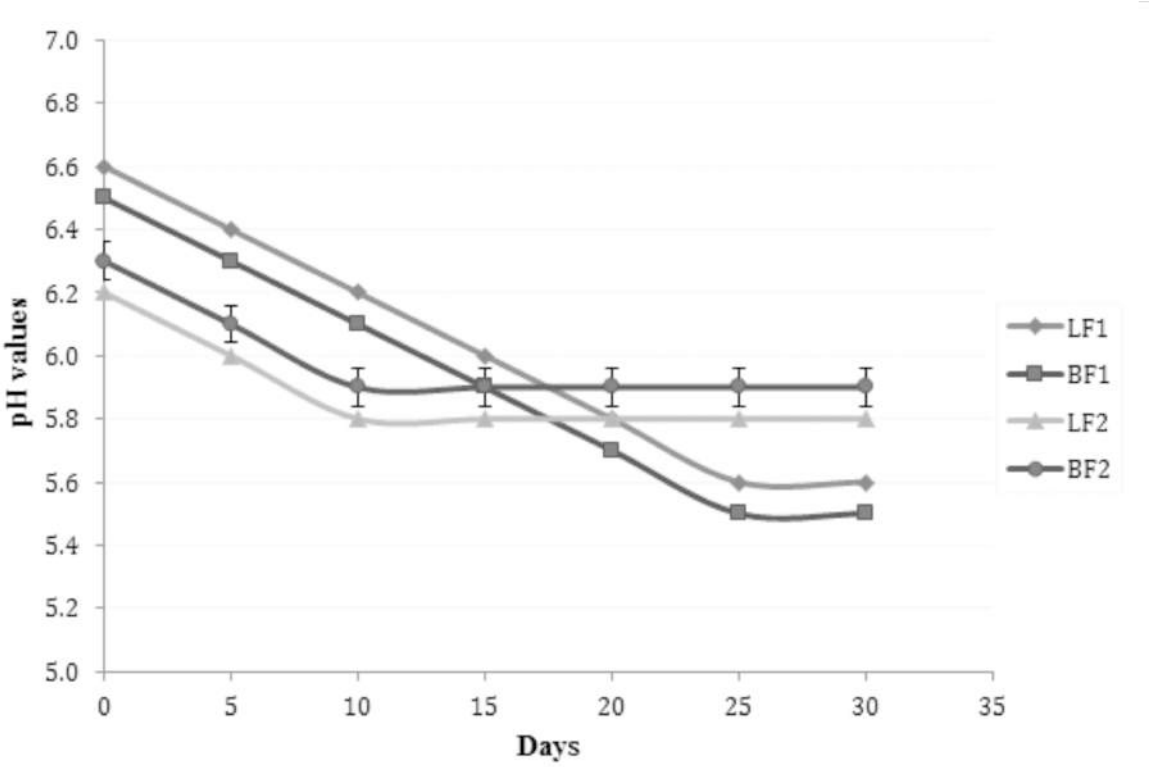

Figure 3: Effect of time on the pH of *L. welwitschii* cream formulations at 25 °C

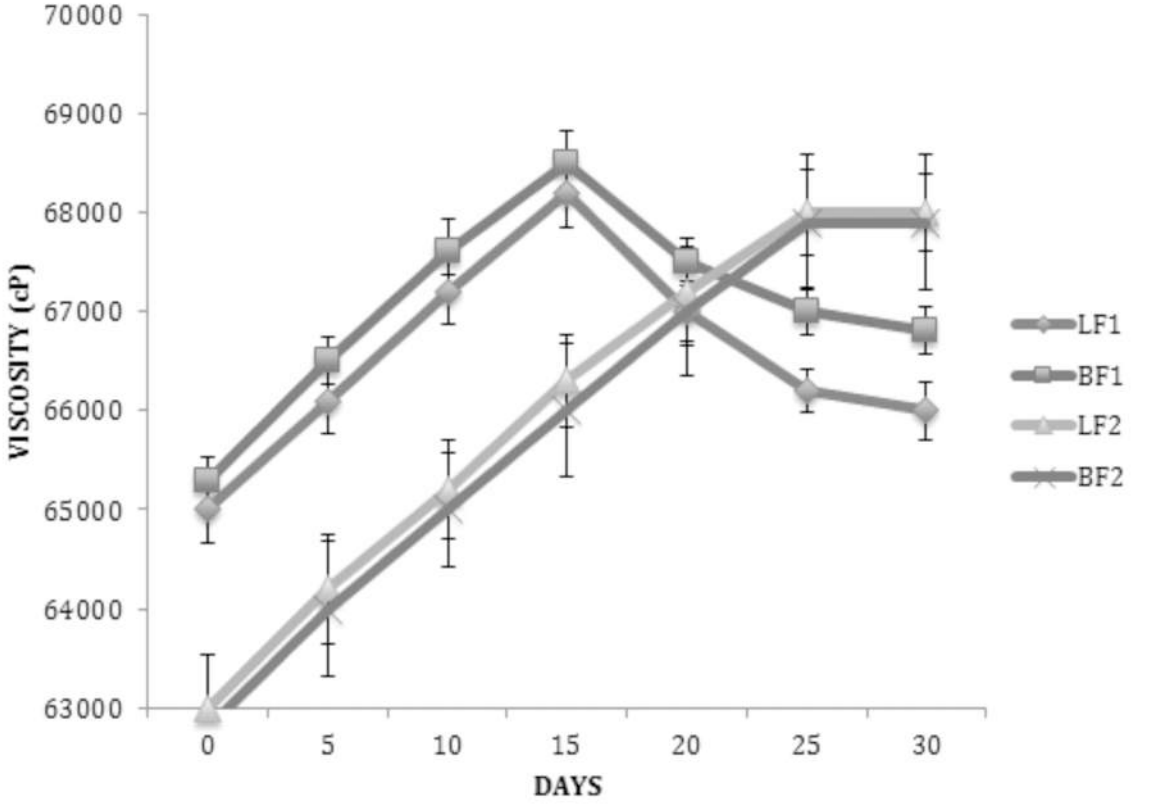

Figure 4: Effect of time on the viscosity of L*. welwitschii* cream formulations at 25 °C

***In vivo* wound healing activity of *L. welwitschii* cream formulations.**

The results obtained from the *in-vivo* assessment of the formulated creams showed that wound contraction was seen to occur gradually until a 95 % result was attained with the leaf extract cream formulation (LF2). The cream formulation (BF2) from the stem bark extract attained 93 % wound contraction activity. Table 4 and Figure 6 presents the percentage wound contraction in comparison to the control and standard formulation.

Table 4: Percentage wound contraction attained by cream formulations of leaf and bark extract of *Lannea welwitschii*

Post-Wounding Days	Percentage Wound Contraction (%)			
	Control	Standard (Cicatrin®)	Cream LF2	Cream BF2
0	0.00	0.00	0.00	0.00
3	11.40	15.39	15.20	18.89
6	17.58	28.54	35.71	34.61
9	24.71	41.23	41.76	41.26
12	33.17	74.07	55.47	48.36
15	52.94	89.27	69.50	61.46
18	71.38	95.15	84.80	77.40
21	84.60	99.53	94.69	92.69

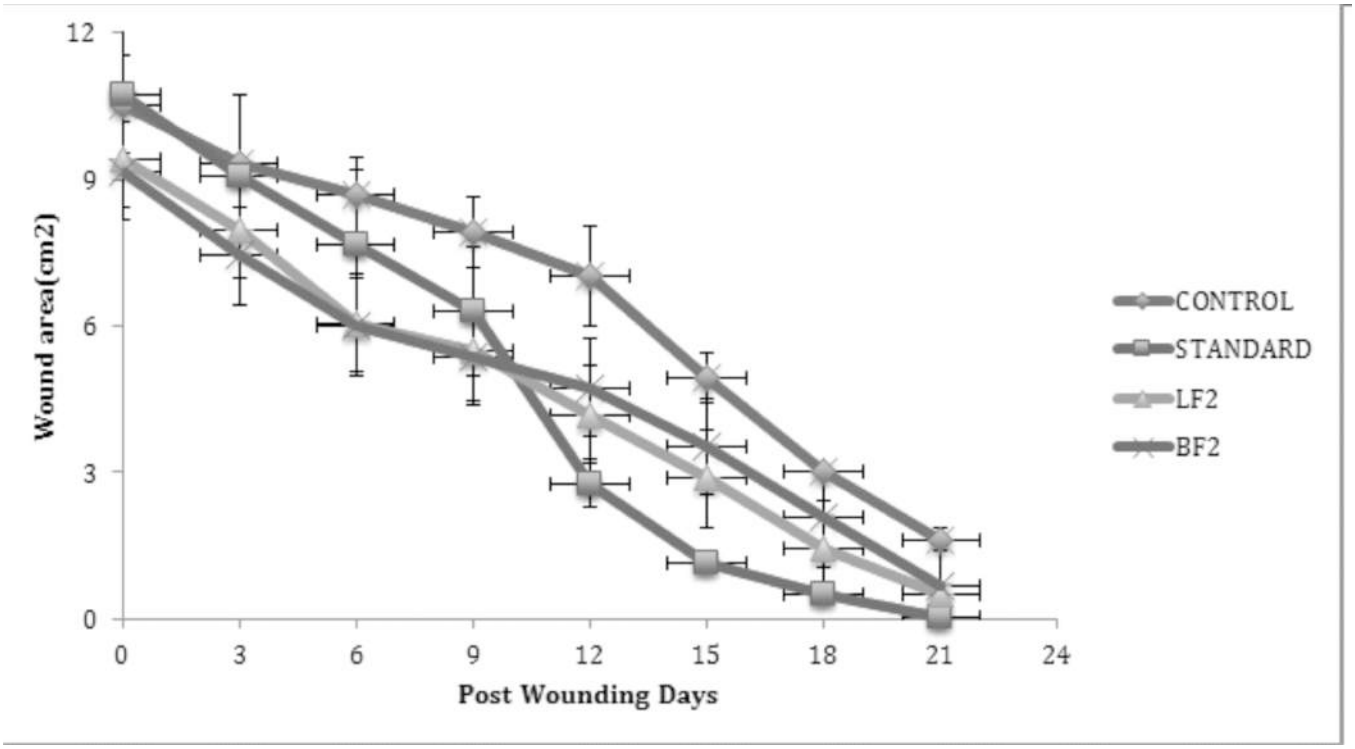

Figure 5: Wound healing activity of the leaf and bark extract cream formulations of *Lannea welwitschii* upon application to excised wounds on albino rats

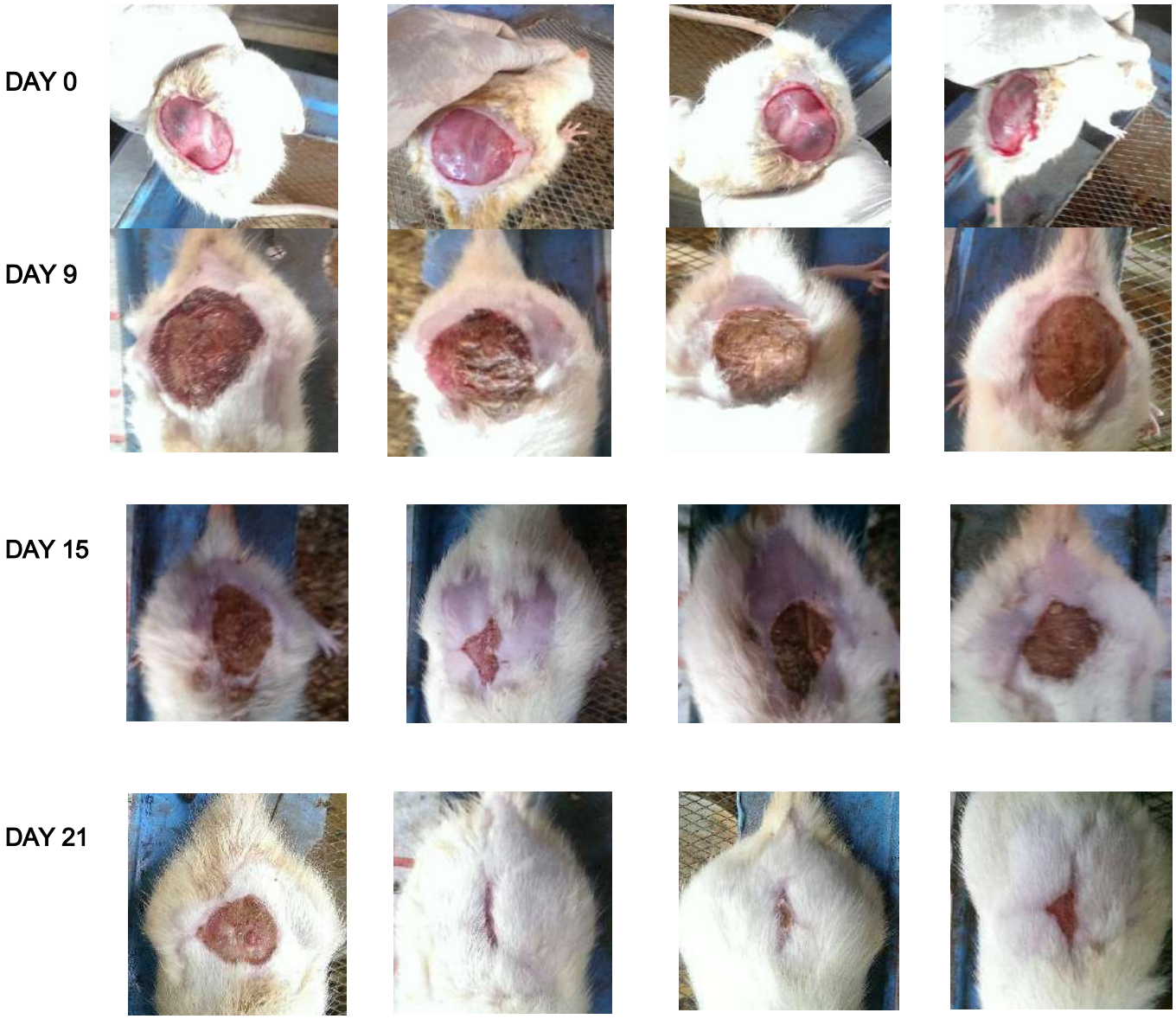

Figure 6: Wound healing activity of leaf and bark extracts of *L. welwitschii* cream formulations over 21days upon application on excised wounds on albino rats

DISCUSSION

Methanol was the solvent of choice for the maceration process because it is known to bring about a high recovery of flavonoids and phenolic compounds from plant materials.[7] Apart from the throughput of extract yield (7.5%w/w and 5.5%w/w for the leaf and bark extracts respectively), other attributes of methanol extraction include low toxicity, ease of evaporation, promotion of rapid physiologic absorption of the extract, preservative action and prevention of the extract to complex or dissociate.[7]

Phytochemical constituents detected in the leaf and bark extract of *L.welwitschii*, include tannins, saponins, flavonoids, cyanogenic glycosides and reducing sugars that have been reported to be secondary metabolites that elicit antibacterial activity observed in the study.[4] The methanol extract showed antibacterial activity against strains of *Proteus mirabilis, Bacillus subtilis* and *Pseudomonas aeruginosa* (microorganisms implicated in various wound and skin infections which in turn delay wound healing processes) but showed no activity against strains of *Staphylococcus aureus, Escherichia coli,* and *Propioni bacterium spp*. This could be as a result of induced genetic mutation in the microorganisms or acquisition of resistant gene from other bacterial species. There was no activity against fungi perhaps because the plant lacks terpenoids and alkaloids that are known to be majorly responsible for antifungal activity in plants.

The MIC obtained for methanol extracts of both the leaves and bark of *Lannea welwitschii* was the same value (4mg/ml). It may be inferred that the chemical structure and composition of the substance responsible for the antimicrobial properties remains intact irrespective of the plant part used and the mechanical processes passed through.

Tween 80 was incorporated in the cream formulation as it is primarily used in cosmetics and beauty products as a surfactant and emulsifier because of its ability to solubilize other ingredients, specifically in the case of oil in water creams[10] while still maintaining the integrity of the active pharmaceutical extract/principle. Upon Storage of the cream at 25°C for 30 days, the integrity of the cream was still maintained and the pH was still within the level of the normal skin pH. Exposure of the creams to high temperature over 30 days indicates the possibility of degradation, as there was a decrease in the pH as well as the viscosity. Hence it is advised that the cream be labeled to be stored in a cool place to prevent irritation as a result of decreased pH as well as problems associated with reduced viscosity which include liquefaction and phase separation.

The formulated creams showed significant wound healing activity compared to the standard drug (Cicatrin®). However, it was observed that the wound contracting ability of the leaves extract in cream LF2 ranks significantly higher than that of the bark extract in cream BF2 ($p<0.05$) with the wound healing potential been evident on day 15. Granulation, collagen maturation and scar formation are some of the many phases of wound healing which run concurrently, but independent of each other. The process of wound healing occurs in four phases (i) coagulation, which prevents blood loss; (ii) inflammation and debridement of wound; (iii) repair, including cellular proliferation; (iv) tissue remodeling and collagen deposition. Any phytochemical which accelerates the above process is a promoter of wound healing. Plant products have been shown to possess good therapeutic potential as anti-inflammatory agents and promoter of wound healing due to the presence of active terpenes, alkaloids and flavonoids.[13, 14] The wound healing activity of *Lannea welwitschii* has been linked to many of its constituents which include tannins, saponins, flavonoids, and antioxidants.[3, 8] Two natural novel cytotoxic alkylated hydroquinone Lanneaquinol and 2(R)-hydroxylanneaquinol have been isolated from the organic extract of the plant *Lannea welwitschii* (Hiern) Engl. of which both compounds exhibited modest cytotoxicity and significant wound healing ability[4] The wound healing property of *Lannea welwitschii* may be due to the presence of these active principles that accelerate the healing process and confers breaking strength to the healed wound. Wound contraction is the process of mobilizing healthy skin surrounding the wound to cover the denuded area. This centripetal movement of wound margin is believed to be due to the activity of myofibroblasts.[15] Since *Lannea welwitschii* extracts enhanced wound contraction, it might have either enhanced contractile property of myofibroblasts or increased the number of myofibroblasts recruited into the wound area.

When comparing cream formulation LF2 and BF2 with commercially available standard drugs frequently used for wound healing, it was observed that most commercially available drugs are either in the powdered form (solid dosage form), ointment (semi-solid dosage form) or liquid dosage-form. The current cream formulation under study would offer an advantage of a good skin or mucous membranes permeation into the underlying layers of the skin. This is as a result of the droplet diameter in topical creams, which generally ranges from 0.1–100 m.[16] It is a more convenient dosage form to apply to a wound area as there is a prolonged contact at the site of application.

The effect of prolonged use of the cream which may be beneficial or associated probably with side effects was not investigated; also, the stability testing on the cream did not exceed thirty days to ensure that its therapeutic activity as well as physicochemical properties are in line with official standards.

CONCLUSION

A non-irritant, stable, oil in water cream formulation from the methanol extract of the leaves and bark of *Lannea welwitschii* formulated for wound healing was developed. The cream formulation demonstrated wound healing potentials as it showed comparable activity with the commercially available standard product in the market. The cream was found to be stable, therapeutically effective and aesthetically acceptable, hence has promising potentials in the pharmaceutical industry.

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