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Development of the Cream Formulation of *Morinda lucida* (Benth) Extracts and Evaluation of its Wound Healing Properties in an Animal Model

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background*: Morinda lucida B*enth (Rubiaceae) commonly known as brimstone has been used in ethnomedicine for wound healing. However, the presentation of this plant in a conventional dosage form for standard dosing and patient acceptance has not been done.

Objectives: This study was designed to formulate the extracts into pharmaceutical cream and investigate its wound healing activity in animal model.

Methods: Leaf ethanol (LE), Leaf aqueous (LAQ), stem-bark ethanol (SE) and stem-bark aqueous (SAQ) extracts of *M. Lucida* were prepared by maceration. Pharmaceutical creams were prepared by standard procedures using 10%w/w extract and assessed by pH, viscosity, extrudability and spreadability. Extracts and creams were evaluated in Wistar rats using incision and excision wound models. For incision, animals were euthanized after 14 days and wound site tissues collected for histology. Excision model was based on wound contraction, closure and epithelialization for 20 days.

Results: The creams had acceptable pH, viscosity, extrudability and spreading times. Histology photomicrographs showed that LE, LAQ and 10% LE cream showed dense, well-arranged and well-positioned collagen, with moderate diffuse cellular dermis infiltration compared to controls. All extracts and cream formulations enhanced wound healing compared to their negative controls while leaf ethanol cream showed optimal activity in significantly (<0.05) reducing wound diameter compared to gentamycin as positive control.

Conclusion*: Morinda lucida* extracts and cream formulations demonstrated significant (<0.05) wound-healing activity in-vivo, in comparison with negative controls and can be improved for translational outcomes.

Keywords: *Morinda lucida*, Extracts, Cream formulation, In-vivo Wound healing, Excision and Incision wound models

INTRODUCTION

The use of medicinal plants mostly in the rural area of the developing countries has always been part of human culture and is wide spread throughout Africa. In Nigeria, a large percentage of the populace depends on herbal medicines because of the absence of modernized functional health facilities or because of the commercially available orthodox medicines are becoming increasingly expensive and out of reach (Ozioma et al., 2019). Plants produce a diverse range of bioactive molecules, making them rich sources of different types of medicines (Abdelrahman & Jogaiah, 2020). *Morinda lucida* Benth**.** (*M. lucida*) is an evergreen shrub that belongs to the madder *family* (*Rubiaceae)*. It is commonly called Brimstone tree, is

popularly called Oruwo in Yoruba (South–Western Nigeria) and among the Togolese it is known as Ewe amaka or Atakake (Ayeni and Aliyu, 2018). Different parts of the plant are used for a variety of purposes across Africa, including dye, flavourings, timber, charcoal, cleaning agents, and so on. *Morinda lucida* contains a variety of chemical compounds that can be used to treat chronic and infectious diseases (Ingle *et al*., 2017; Chakraborty *et al*., 2022). Preliminary phytochemical screening of different extracts prepared from the bark, leaves, and seeds of *M. lucida* revealed the presence of Saponin, light Tannins, Alkaloids, Flavonoids, and Cardiac glucoside. It also confirms the presence of minerals such as sodium, potassium, calcium, iron, zinc, copper, and phosphorus. *Morinda lucida* has been used as a reducing agent in the synthesis of silver nanoparticles and the outcome demonstrated antioxidant and antimicrobial activity (Labulo et al., 2022). Oladeji et al., (2022), reported the antiplasmodial activity of the leaf and bark extracts against Plasmodium berghei infected mice. Adewole et al., (2021) have demonstrated the trypanocidal activity of *Morinda lucida* through ethnopharmacological reviews of several studies. Adeneye et al., (2017) investigated the modulatory effect of the aqueous stem bark extract on blood glucose and lipid profile in alloxan-induced diabetic rats and reported favourable outcome. The wound healing activity of *Morinda lucida* has been reported by previous studies (Adeleye et al., 2018 and Adewole et al., 2021). In addition, the root extracts have demonstrated *in vitro* activity against multi-drug-resistant bacterial pathogens isolated from diabetic foot ulcers, thereby facilitating healing (Achukwu et al., 2024).

Wound is a tear, piercing, cut, or other break in the skin or an external surface. Wounds, according to the Wound Healing Society, are physical injuries that result in an opening or break in the skin, causing disruption in normal skin anatomy and function (Han, 2023). Wounds not only harm the physical and mental health of millions of patients, but also cost them a lot of money. Wound infection is one of the most common diseases in developing countries because of poor hygienic conditions (Sen, 2019). Current estimate indicated that nearly 6 million people suffer from chronic wounds and it has been the major causes of physical disabilities (Sen, 2021).

Pharmaceutical creams are topical preparations with many advantages over oral formulations such as ease of application, large surface area for medication, selective release, and suitability for self-medication and reduction in frequency of use thereby increasing patient compliance (Sahu et al., 2016). The risks and inconveniences of intravenous drug delivery are also circumvented by the use of topical medications like cream (Palmeira-de-Oliveira et al., 2022).

Although some studies have reported the wound healing effect of *Morinda lucida* but none has attempted the formulation into a dosage form. This study therefore explores the formulation of its extract into pharmaceutical cream and evaluate the wound healing properties using excision and incision models. Since bioactive agents with antioxidant activity are satisfactory for wound healing, the antioxidant potentials will first be studied before formulation. It is hoped that the extract and cream formulations will offer wound healing activity while the formulation would make available a standardized and acceptable delivery system available for possible translational outcome instead of just recommending the extracts for patient use.

METHODOLOGY

Plant Collection, Identification and Authentication

Plant materials (Leaves and Stem bark) was collected at Unity Community Resident beside Aba Omo, along All Saint College Road Apata, Ibadan. It was identified and authenticated in University of Ibadan Botany Herbarium. A voucher specimen (Voucher no. UIH-23217) was deposited in the Department of Botany Herbarium, University of Ibadan (DBHUI).

Preparation of the plant extracts

The plant materials (leaves and stem bark) collected were washed under running tap water to remove adhering materials and the stem bark were cut into smaller pieces before it was air-dried under ambient condition, then the dried plant materials (leaves and stem bark) were pulverized using a laboratory milling machine (Fritsch, Germany). The leaves were extracted by macerating 400 g of the powdered leaves in 3.5 L of distilled water for 24 hours while 450 g of the powdered leaves was macerated in 5 L of 95 % ethanol for 72 hours. The pulverized stem bark was also divided, 350 g were macerated in distilled water and 1000 g were macerated in 95 % ethanol for 72 hours. The aqueous and the ethanol extracts were filtered with Whatmann No.18 filter paper and the filtrates were collected. The ethanol and aqueous extracts were concentrated using rotary evaporator (Corning, USA) and the prepared extracts were collected and air dried to fully remove the extracted

solvents. The extracts were stored at room temperature in airtight containers prior to use.

Phytochemical screening of extracts

All the prepared extracts were evaluated for presence of various phytoconstituents by performing different qualitative chemical tests according to established procedures.

Antioxidant capacity of extracts

The antioxidant capacity (AOC) of extract was determined *in vitro* using the DPPH (2,2-diphenyl-1-picryl- hydrazyl-hydrate) assay (Bassaran et al., 2022). The AOC was carried out with 50 µL of the extracts added to 2 mL of DPPH dissolved in methanol. The changes in absorbance were analyzed at 517 nm using UV–vis spectroscopy (UV-1700 PharmaSpec, Shimazu Deutschland, GmbH, Germany). The blank used was 2 mL of 0.1 mg/mL DPPH in methanol and AOC was expressed as% inhibition of DPPH. The calculation of AOC was done using Equation 1

$$DPPH\ (\%) = \{(A - B)/A\} \times 100 \quad \text{……..Equation 1}$$

Where, A is the absorbance of the blank at 517 nm and B is the absorbance of the sample at 517 nm **(**Adegbolagun *et al.*, 2018).

Cream formulation

The base (aqueous cream BPC) was prepared according to established method (Aponjolosun et al., 2023). Chlorocresol (0.1 % $^{w}/_{w}$) was dissolved in warm distilled water (69.9 % $^{w}/_{w}$) and then mixed with hot melted ointment; 30 % $^{w}/_{w}$) and was continuously stirred for a while until its cold. Propylene glycol, (10.0 %$^{w}/_{w}$) was mixed with appropriate quantities (5 and 10 %$^{w}/_{w}$) of plant extract and incorporated into the base respectively by continuous stirring, until pleasant products were formed. The choice of the 10% cream formulation for animal studies was based upon the release properties of the cream formulations. The 5% had faster release rates that seemed like a burst release which is not desirable as semi solid formulations are expected to demonstrate prolonged release of bioactive agents. The choice of 10% for propylene glycol is due to our past studies. The concentration of humectant should be moderate between 5 to 10 %. Anything greater may move it from being an additive to an active agent with antimicrobial properties.

Physicochemical properties of the cream formulations

The organoleptic properties were visually observed, the pH of the cream formulations was measured at 27 ± 2 $^{\circ}$C using a pH meter (Jenway Model 3520, Essex, UK). Spreading time was done by placing 0.5 g of each formulation on a previously cleaned slide and covered with another slide. Weight (1 kg) was placed on the covered slides for 5 min for even spread. The spreading time was determined by the length of time taken to separate the two slides. The viscosity of the formulations was carried out using a Brookfield viscometer (VT 181, Karlsruhe, Germany) at 27 ± 2^{0}C using spindle number seven and a rotational speed of 100 rpm. The rheological pattern was obtained by using different shear rates 2.5, 4, 5, 10, 20, 50 and 100 rpm, to measure the viscosity and appropriate plots were then made. The extrusion time was determined with pre-weighed syringes filled with 2 mL of cream formulations and extrusion was done for 1 min and amount extruded was obtained for each cream.

Experimental animals

All animals {Wistar albino rats, (*Rattus norvegicus*)} of either sex (150-240 g) used in this experiment were obtained from the Animal House of the Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Ibadan, Nigeria. The animals were housed individually in standard cages and kept at 26 ± 2ºC and relative humidity of 44-56%, they were also allowed to acclimatize for 7 days before the commencement of the experiments. They were freely maintained on standard rat pellets (Chinkun growers feeds, Ibadan, Nigeria), and was given water *ad libitum*.

Acute Toxicity Study

Eight (8) groups of the acclimatized rats were weighed separately and placed back in their cages, extracts were given in different concentrations- 10, 100, 140, 225, 370, 600 and 1000 mg/kg of the leaf and stem bark extracts respectively. Another group was given normal saline to serve as control. The rats were observed for 24 hours for all symptoms of toxicity and mortality. In addition, all animals were observed hourly on the day of extract administration, and survived animals were monitored daily for 2 weeks for physical signs of toxicity such as writhing, decreased motor activity, decreased body/limb tones, decreased respiration and finally death. Records on the number of deaths observed were taken in each group daily. The LD50 was calculated as the geometrical means of the

maximum dose producing 0% and minimum dose producing 100% mortality.

Wound healing experimental models

The incision models had fifteen groups but animals were kept in separate cages to prevent partner grooming that could affect outcome. After anaesthesia, two paravertebral long incisions were made through the skin and cutaneous muscles at an average distance of about 1.3 cm from the midline on each side of their depilated back as described by Dash *et al.,* (2009). The parted skin was kept together by stitching with a 2/0 nylon surgical thread and curved needle at an interval of 0.5 cm and continuous threads on the both incised edges were tightened for good wound closure (wound contraction). The histological text was carried out after the experiments. The rats in each group were sacrificed by euthanizing them after which all tissues of the rats according to their groups were collected in sample bottles, labelled and their carcass were buried.

For the excision model, the rats were anaesthetized prior to and during the creation of wounds with 1ml of intravenous ketamine hydrochloride (10 mg/kg body wt.). It followed the procedure of Rashed *et al*., (2003) and Nagapa *et al*., (2001) with slight modification. The animals were also randomized according to the distribution in Table 3. Each rat was anaesthetized as previously described above. Hair was removed by shaving the nape of the back of all the rats. Ethanol (70%) was used as antiseptic for the shaved region before making the wound. A full thickness of the excision wound of an average of 1.5 cm in diameter circular area were created along the markings using appropriate device. The wounds were left undressed to the open environment and no local or systemic anti-microbial agents were used.

The rats used for both wound models were topically treated once daily starting from the wound creation onset till end of the study.

Statistical analysis

Results were presented as mean ± standard deviation (mean ± SD) at $p < 0.05$. Prism Software (Graph pad) package 8.0 was used to analyse relevant data at $p < 0.05$ using one way analysis of variance (ANOVA).

RESULTS

The results from the phytochemical screening of the bioactive compounds present in both the ethanol extracts and aqueous extracts of the leaf and stem bark of *Morinda lucida* are presented in Table 1. It shows that the stem bark and leaf ethanol extracts contain eleven (11) bioactive compounds which includes Saponin, Alkaloids, Flavonoids, Tannin, Coumarin, Steroid Terpenoid, Cardiac Glycosides, Quinones, Phytosteroids, and Phenols but Glycosides, and Anthocyanin were absent whereas the leaf and stem bark aqueous extracts contain ten (10) of the above bioactive compounds but cardiac glycosides, and phytosteroids were absent.

The acute toxicity of the extracts showed that no mortality was recorded in any of the groups during the experiment, there was also no weight loss across the groups, the animals were as mobile as the control, there was also no reduction in their feeding, and no lethality in any group of the experimental rats at a dosage greater than 1000 mg/kg body weight after oral administration of different doses.

The ingredients for preparing cream formulations are detailed in Table 2, Table 3 shows the animal randomization into groups for all experiments, while Table 4 shows the physicochemical attributes of the cream formulations. The properties of the cream formulations were within acceptable standards for semi-solid formulations. For example, the viscosities were high, a parameter which confers prolonged activity on the cream. Furthermore, the pH of the formulations was within acceptable limits (4-6) for skin preparations. The spreading times were less than 5 minutes and it shows the ease of use for patients when applying to the skin. Generally, there were no particular trends in the properties of the cream formulations except for the t80, a release parameter. The cream formulations containing 5% extract had faster release (about 90 min) while the 10 % formulations showed expected prolonged release. The 10% cream formulation was then chosen for the animal experiment since creams are generally used once daily. The treatments were also administered once daily. Figure 1 describes the antioxidant activity of the extracts in comparison to ascorbic acid. The antioxidant activity was concentration-dependent. It shows promise for wound healing, since antioxidation helps in wound healing.

Table 1: Phytochemical constituents of leaf and stem bark extracts of *Morinda lucida*

Phytoconstituents	Stembark aqueous extract	Leaf aqueous extract	Stembark ethanol extract	Leaf ethanol extract
Saponin	+	+	+	+
Alkaloid	+	+	+	+
Flavonoid	+	+	+	+
Tannin	+	+	+	+
Coumarin	+	+	+	+
Steroid	+	+	+	+
Terpenoid	+	+	+	+
Cardiac Glycosides	-	-	+	+
Glycosides	-	-	-	-
Quinones	+	+	+	+
Anthocyanin	-	-	-	-
Phytosteroids	+	+	+	+
Phenols	+	+	+	+

Table 2: Details of ingredients in the cream formulations

Formulation Description	Aqueous cream BPC (%)	Propylene Glycol (%)	*M. Lucida* Extract (%)	Total (%)
Stem bark ethanol cream	85.0	5.0	10.0	100
Stem bark aqueous cream	85.0	5.0	10.0	100
Leaf ethanol cream	85.0	5.0	10.0	100
Leaf aqueous cream	85.0	5.0	10.0	100
Stem bark ethanol cream	80.0	10.0	10.0	100
Stem bark aqueous cream	80.0	10.0	10.0	100
Leaf ethanol cream	80.0	10.0	10.0	100
Leaf aqueous cream	80.0	10.0	10.0	100

Table 3: Experimental distribution of animals

Group No	Identification	Treatment administered
1	Negative control I	Normal saline
2	Standard	Gentamycin
3	Negative control II	Aqueous cream BPC
4	Extract I	Ethanol leaf extract
5	Extract II	Ethanol stem-bark extract
6	Extract III	Aqueous leaf extract
7	Extract IV	Aqueous stem-bark extract
8	Cream I	Formulated cream containing 10 % ethanol leaf extract
9	Cream II	Formulated cream containing 10 % aqueous leaf extract
10	Cream III	Formulated cream containing 10 % Ethanol stem extract
11	Cream IV	Formulated cream containing 10 % aqueous leaf extract

**The randomization was used both for the excision and incision models making 22 groups in all*

Table 4: Physicochemical properties of the cream formulations

Formulation name	Extract concentration (%w/w)	Extrudability (mL/min)	Spreading time (min)	pH	Viscosity (cP)	t_{80} (min)
Leaf ethanol cream	5.0	13.27±1.51	3.65±0.21	4.31±0.15	18000 ± 113.7	91.55
Stem ethanol cream	5.0	8.85±0.35	4.2±0.14	4.79±0.20	7200 ± 565.7	79.21
Leaf aqueous cream	5.0	10.4±0.85	2.70±0.14	3.89±0.30	12250 ± 777.8	89.78
Stem aqueous cream	5.0	14.45±0.78	2.55±0.35	5.69±0.01	7100 ± 572.8	75.67
Leaf ethanol cream	10.0	10.4±0.85	2.80 ± 0.14	4.79±0.20	18400 ± 131.4	299.28
Stem ethanol cream	10.0	7.53±0.88	4.90 ± 0.14	5.59 ± 0.04	7200 ± 204.1	215.72
Leaf aqueous cream	10.0	10.65±0.49	3.40 ± 0.28	4.40±0.12	15200 ± 433.1	263.9
Stem aqueous cream	10.0	13.9±1.56	2.65 ± 0.21	6.04±0.08	20000 ± 613.7	325.22

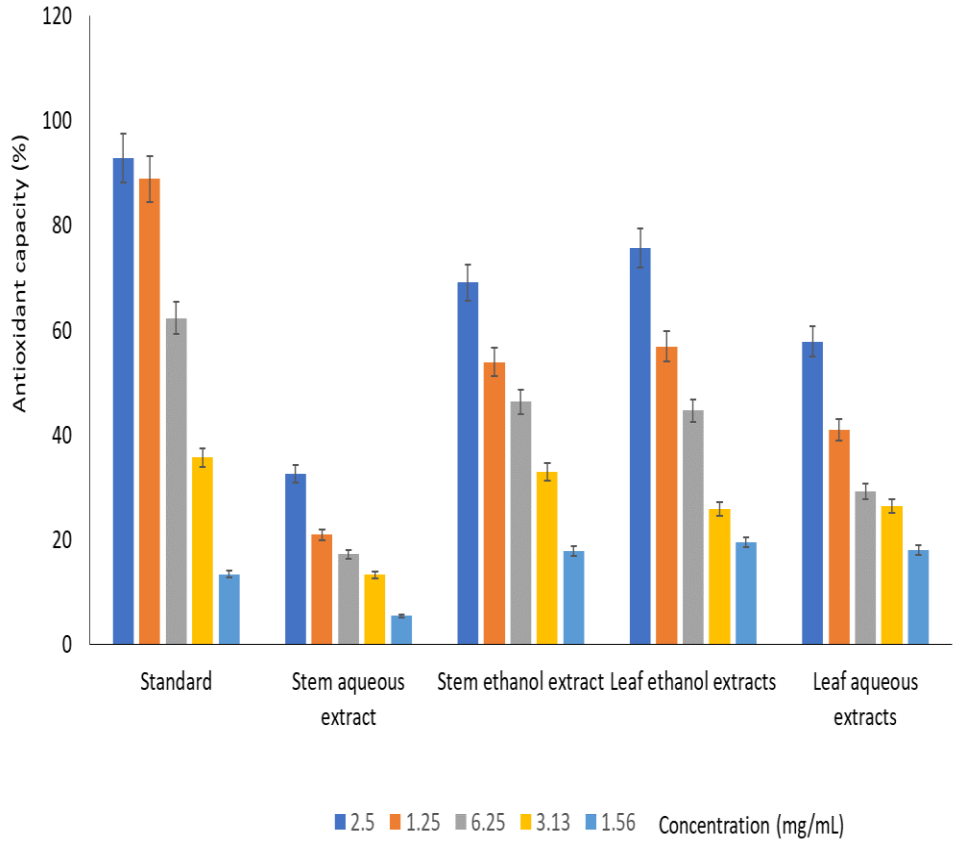


Figure 1: The antioxidant activity of *Morinda lucida* extracts

The wound healing of the extracts and cream formulations were presented in Figures 2 and 3 respectively. The results showed that all extracts demonstrated wound healing activity as wound sizes reduced as days of treatment increased. Figure 4 is a comparative plot of the activity of extracts and cream formulations while Table 3 is the outcome of its statistical analysis. Figure 4 showed that the cream formulations presented higher wound healing properties because at 10 % extract composition only,

the leaf ethanol cream had reduced wound length by 96.6 % compared with the extract (100 %) which achieved 96.4 % reduction. The activity of the cream formulations is significantly ($p<0.0001$) higher than that of the extracts and base. Figure 5 shows typical animals treated during the experiments and the macroscopic outlook of the wounds as healing progressed.

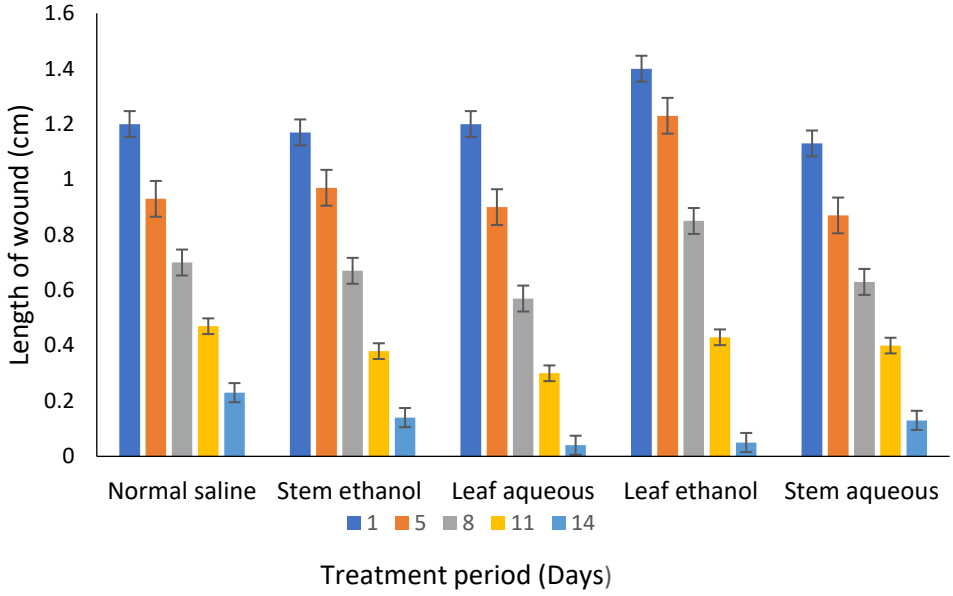


Figure 2: The wound healing activity of the extracts compared to normal saline

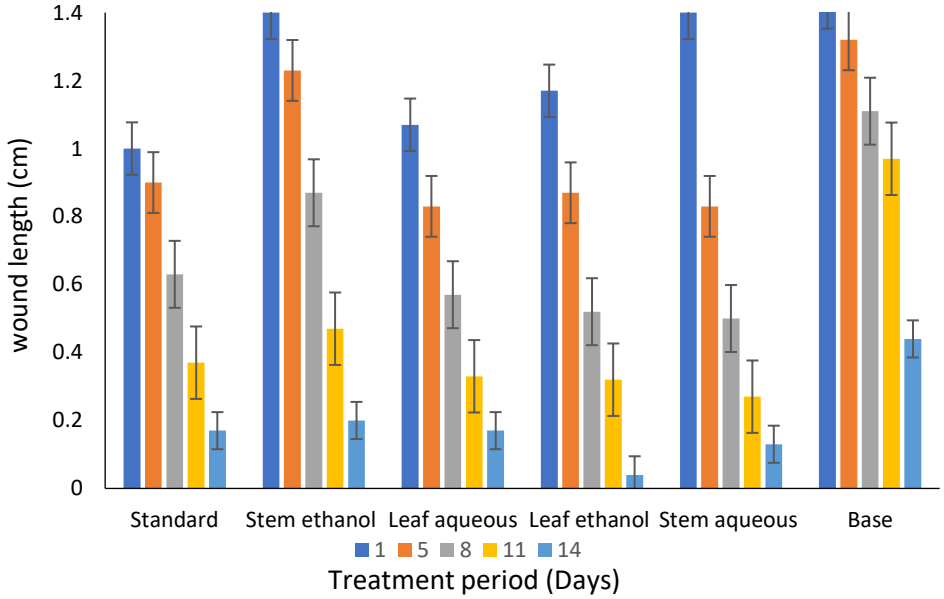


Figure 3: The excision wound healing activity of the cream formulations compared to the standard

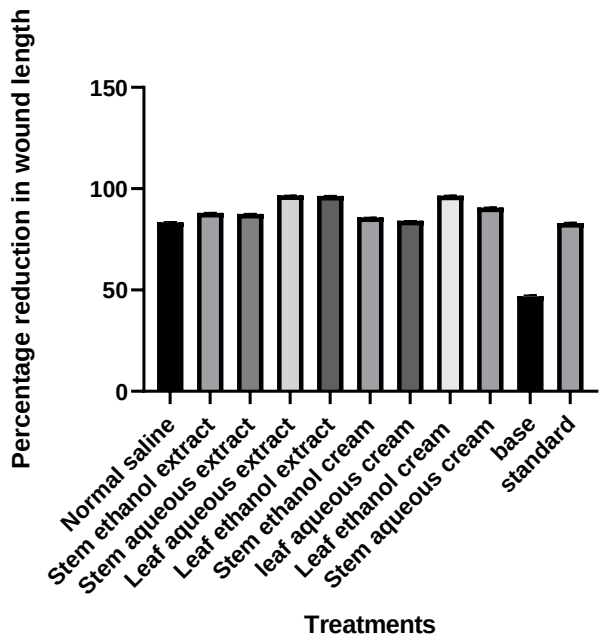
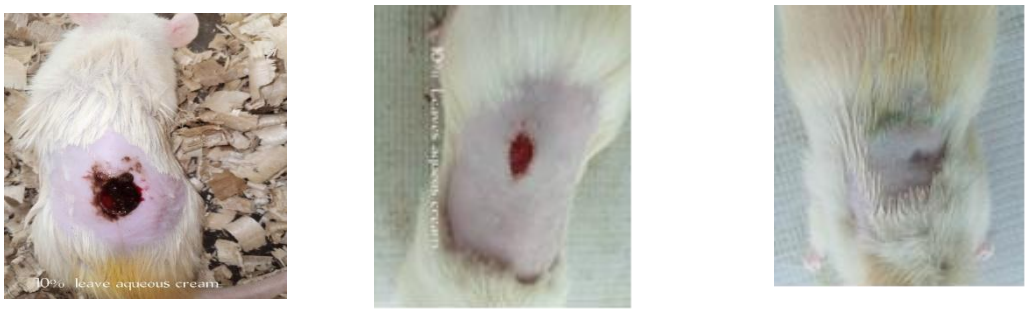


Figure 4: Comparative activity of extracts and their formulated creams in incision wound reduction



Incision at Day 5 Reduced incision at Day 11 Incision almost invisible at Day 14

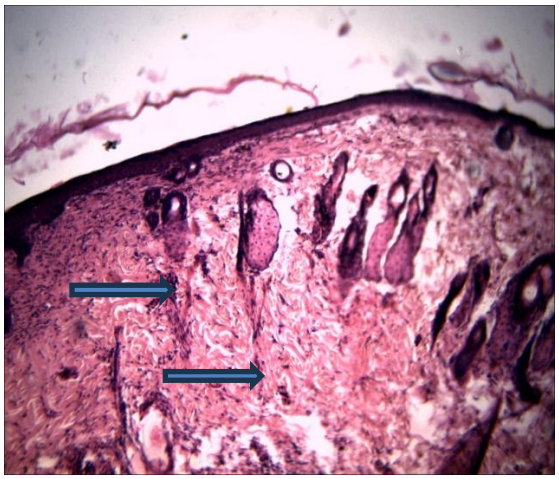
A

Incision at Day 5 Reduced incision at Day 11 Incision almost invisible at Day 14

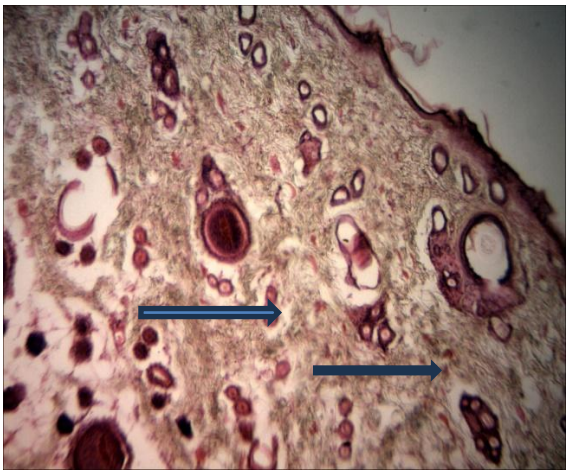
B

Figure 5: The incision wound healing progression of animals treated with A-Aqueous extract B-Ethanol extract

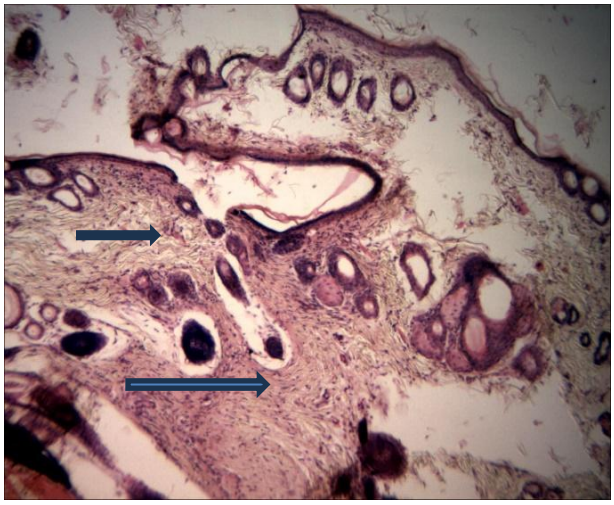
According to the histology reports shown in Figure 6, follicles and glands were abundant and prominent in animals treated with the cream formulation of *M. lucida* leaf ethanol extract. The collagen is also dense as seen in the arrowed areas. The leaf ethanol extract of *M. lucida* also produced dense and regularly arranged dermal collagen in the animals as shown through the arrows. In the normal saline-treated animal, collagen fibres were very scanty within the dermis as shown by the arrows, and glands were present. For the standard cream, follicles and dermal glands were also very prominent.



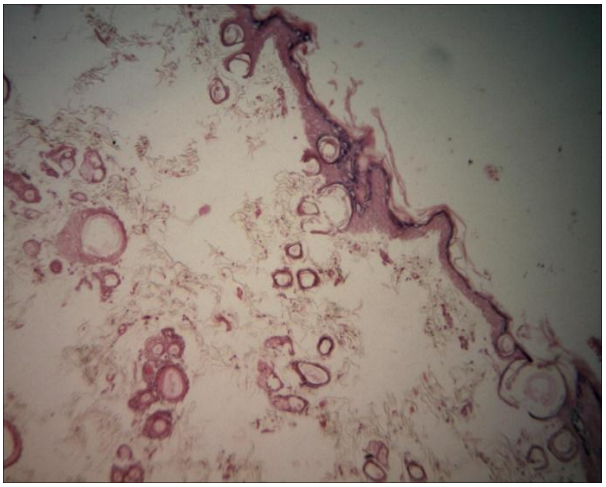
Leaf ethanol extract: Dense regularly arranged dermal collagen (arrows)



Standard: Follicles and dermal glands are very prominent



Leaf ethanol cream formulation: Follicles and glands are abundant and prominent. The collagen is also dense (arrows)



Normal saline: Collagen fibres very scanty within the dermis (arrows) glands are present

Figure 6: Histology showing the healing activity of controls, leaf ethanol extract and its cream formulation on incision wound

DISCUSSION

It is well known that plants contain chemical substances known as phytochemicals. Some of these substances have both pharmacological and other bioactive potentials. Plants have been used for ages for a variety of human-beneficial purposes, including the treatment of illnesses. One of these plants with several pharmacological advantages is *Morinda lucida* and one of such benefit is in the treatment of wounds (Idih *et al*., 2021). The phytochemical screening reveals bioactive compounds present in both the ethanol

extracts and aqueous extracts of the leaf and stem bark of *Morinda lucida.* The findings in this study shows that the ethanol extract and the water preparation of leaves have a potential benefit in enhancing the wound healing process. This observation corroborates its use in traditional medicine in the treatment of wounds. The results of phytochemical screening obtained from extracts of this study agree with those previously reported by Ogundare and Onifade, (2009) and Patience *et al*., (2010). Flavonoids are known to inhibit specific enzymes and as well protect the vascular system and strengthen the tiny capillaries that carry oxygen and essential nutrients to all cells aiding in wound healing. Of course, this is also a polyphenol function (Farooq *et al*., 2022). These active constituents promote the process of wound healing by increasing the viability of collagen fibrils, by increasing the strength of collagen fibers either by increasing the circulation or by preventing the cell damage or by promoting the DNA synthesis (Barku *et al*., 2018).

Previous reports from (Idih *et al*., 2021) states that ethanol extract of *M. lucida* leaves possess more phytochemicals of pharmacological relevance in medicines. Wound healing process is often characterized initially by coagulation, to control excessive blood loss from the damaged vessels. Following this, the wound becomes inflamed and debrided, and then the skin regenerates its epithelium by proliferating, migrating, and differentiating its squamous epithelial cells. In the final stage of the healing process, collagen deposition in the dermis and remodeling occurs (Builders *et al*., 2021) which is also a function of polyphenols (Progression of healing is usually seen as reduction in surface area by contraction which is characterized by mobilization of healthy skin surrounding the wound to cover the denuded area.

The outcome of the toxicity trials is an indication that the extracts may be safe for human consumption, confirming the belief of herbalists that *Morinda lucida* leaf and stem bark is not harmful and it is thus safe for human consumption. Although the cream formulations produced in this study are for external use, the conduct of the acute toxicity is to have a window to the safety of the plant extract in case by penetration, the cream can get beyond the skin. Dermal toxicity is common when plant extract formulations exhibit pH beyond the skin tolerable level. Since the pH of the cream formulations were satisfactory, they can find usefulness for patients at this stage of the study.

The antioxidant activity and percentage inhibition showed that the leaf ethanol extract had the highest percentage of inhibition of 75.68% at 0.25 mg/mL and 56.91% at 0.125 mg/mL followed by the Stem ethanol extract (Builders *et al*., 2021). The result of acute toxicity test aligns to the fact that there wasn't any death in any of the groups confirming that there is no mortality in any of the groups during the experiment, showing that both the aqueous and ethanol extracts of the leaf and stem bark extracts of *M. lucida* is not toxic or harmful. There was no lethality (LD_{50}) in any group of the experimental rats at a dosage greater than 1000mg/kg body weight after oral administration of different dosage of the extracts according to their respective body weight. This is an indication that the extracts may be safe for human consumption, confirming the belief of the herbalists that *Morinda lucida* leaf and stem bark extracts is not harmful and it is safe for humans (Nworu *et al*., 2012). The acute toxicity test (LD_{50}) after oral administration of 2000 mg/kg body weight of the extracts revealed no toxicity at this dose as stated by other researchers. There were no significant alterations in water or food consumption, or body weight during the experiment (Nworu *et al*., 2012). This is also in line with a study that was done to figure out the LD_{50} of this extract, and the results showed that it was safe because no deaths had ever been reported at doses as high as 500 0mg/kg.

According to Aponjolosun et al., (2023), oil-in-water emulsions are the most commonly used cosmetic delivery systems because they provide moisture to the skin while also improving its condition by forming an occlusive obstruction on it. Humectants are hydrophilic substances that are added to aqueous phases to absorb water and prevent degradation. The humectant (propylene glycol) improved the formulation's lustre. A formulation's pleasant color can improve patient adherence to medication. This study found out that the formulated creams have good spreading values, which could be attributed to the presence of the base and humectant. The spreading property of a formulation also influences its bioavailability efficiency. Furthermore, semisolid formulations should spread easily without generating much drag or friction during the rubbing process. According to Ajala et al., (2016), creams with pH values ranging from 4 to 6 are compatible with the skin and would not negatively interfere with the integrity of the skin. The high viscosity of the cream would enhance their adherence to the skin thereby promoting their prolonged presence at a site of application.

This study confirmed the potency of the ethanol and aqueous extracts of *Morinda lucida* benth and its cream formulations, the reference parameters are contraction of the wound area and epithelialization (restoring the epidermis). Wound contraction facilitates healing in a short time as it decreases the size of the wound and reduces the amount of extracellular matrix needed to repair defected tissues.

Contraction also facilitates healing by promoting epithelialization. Epithelialization (re-epithelialization) is achieved by epithelial cells' (keratinocytes') migration from the basement membrane upward or from the wound edge. The leaf and stem ethanol extracts has the highest wound healing activity when compared to the control. They both completed the wound contraction and period of epithelialization earlier than the controls and other extracts. This goes further to establish the forkloric claim of *M. lucida* as having wound healing capacity. This can be attributed to its richness in important bioactive compounds like flavonoids, phenols, anthraquinones etc. high presence of all macro nutrients (Osuntokun et al., 2016).

Histology revealed that the concentrated leaf ethanol extracts and the leaf ethanol cream formulation accelerate wound healing by enhancing epithelialization and collagen deposition, a remarkable resolution of wound healing. The collagen was very dense, well-arranged and well positioned. The stem ethanol extracts and its cream formulations reveals that the collagen fibres are sparsely distributed with the hair follicle sections been prominent. Both features characterize a wound healing process as a resolution of inflammation resulted from the high activity of flavonoids and polyphenols. This result suggested another avenue for

combinatorial therapy where phenols and flavonoids can be used to enhance the efficacy of conventional antibiotics. The extracts and cream formulations exhibited wound healing properties in both incision and excision models.

ETHICAL CONSIDERATIONS

All animal experiments followed national and institutional guidelines in accordance with the revised National Institute of Health standards for the Care and Use of Laboratory Animals (Grosblatt, 1978). The ethical approval for animal use was also obtained from the University of Ibadan Animal care and Use Research Ethics Committee (UI-ACUREC/App/2023/040).

CONCLUSION

The leaf and stem bark extracts of *Morinda lucida* had relevant phytochemicals with antioxidant potential which are helpful in wound healing. The extracts demonstrated wound healing in both incision and excision models. The cream formulations had acceptable pH in the skin range and high viscosity which would enable prolonged action on the skin. The cream formulations showed wound healing in both models used. The cream formulations presented higher wound healing properties because at 10 % extract composition only, the leaf ethanol cream had reduced wound length by 96.6 % compared with the extract (100%) which achieved 96.4 % reduction. In comparison to the cream base, all cream formulations offered significantly higher wound healing activity in both models. Summarily, *Morinda lucida* extracts and its cream formulations had acceptable physicochemical properties and demonstrated wound healing in both incision and excision wound models.

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