Research Article

The Wound Healing Effect of an Ointment Formulation Containing *Baphia nitida* on Adult Wistar Rats

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ABSTRACT

Phytomedicines are safe, potent, available and affordable. They possess many active constituents with many pharmacological activities such as antibacterial, antifungal, antioxidant, etc. The objectives of the research work are to formulate and evaluate the wound healing property of methanol extract of *Baphia nitida (Fabaceae)* leaves. The extract was formulated as an ointment dosage form and evaluated for its wound healing potential activity in Wistar rats using the contraction of excision wound model. The phytochemical screening was carried out on the methanol extract. The extract at different concentrations (5 %, 10 %, and 20 %) and 5 % Cicatrin® powder (a standard reference) were formulated as ointments. The phytochemical tests indicated that the methanol extract was found to contain alkaloids, flavonoids, saponins, terpenoids, and tannins. The optimum dose (10 %) had faster wound area reduction than the standard reference, Cicatrin® though not significant. The optimal concentration significantly varied ($p < 0.05$) from 5 % extract, and the placebo ointments. There was a progressive decrease in the wound area with time in all the formulations at different rates. The wound healing activity of *Baphia nitida* was found to be dose dependent as the ointment containing 10 % of extract showed complete epithelization within day 13 post-surgery, while at 20 % concentration, it was observed that there was poor adsorption of extract into the ointment base that resulted in lesser activity. Therefore, this study revealed that *Baphia nitida* has intrinsic wound healing properties at a given concentration (10 %).

Keywords: Wound, methanol extract, excision wound, *Baphia nitida*.

INTRODUCTION

Research is increasing for medicinal plants and its ingredients as an alternative source of medication to orthodox medicine, especially in developing countries. Phytomedicine has been a source of medicinal treatment for many years. Phyto-products are safe, potent, available and affordable. They possess many active constituents with many pharmacological activities such as antibacterial, antifungal, antioxidant, etc. For this, new herbal formulations will be potentially available for therapeutic utilization.

A wound means a defect or a cut in the skin resulting from the physical or thermal damage or as a result of the presence of an underlying medical or physiological condition.[1] Wound Healing Society describes wound as a disruption of normal anatomic structure and function.[2] The wound could be classified as acute or chronic wounds. An acute wound is usually tissue injuries that heal completely with minimal scarring within the expected time frame, usually 8-12 weeks.[3] Chronic wounds arise from tissue injuries that heal slowly; they take beyond 12 weeks to heal and often may reoccur.[4]

This kind of wounds fails to heal due to repeated tissue insults or underlying physiological conditions such as diabetes and malignancies, persistent infection, poor primary treatment, and other patient-related factors.[5] There are other factors that could influence wound healing such as nutritional deficiency, drugs, sterility, obesity, movement of wound edges and site of the wound.[6] Also, a wound was described as both acute and chronic and wounds that are difficult to heal as complex wounds with unique characteristics.[7] The properties of complex wounds include extensive loss of the integument which comprises skin, hair and associated glands; infection such as Fourier's gangrene which may result in tissue loss; tissue death or signs of circulation, impairment, and presences of pathology. A wound should be properly handled to avoid pathologic state intervention. Wound infections are more ramparts in developing countries due to the poor hygienic environment. The microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyrogenes*, *Streptococcus pneumonia, Pseudomonas aeroginosa*, and *Klebsiella pneumoniae* cause wound infection.[8] Bacterial infection is a major factor affecting wound healing. Bacterial directly invade wound producing inflammation and fluid exudates, which interferes with healing and its toxins causes tissue damage and delays fibroplasias as well as collagen synthesis.[9]

Wound healing involves a highly dynamic integrated series of cellular, physiological and biochemical processes, which occur in living organism.[10] Repair through regeneration is very common in unicellular and the lower metazoan animals but highly restricted in the higher animals.[11] Wound healing is a specific biological process related to the general phenomenon of growth and tissue regeneration. Wound healing process involves some steps such as granulation, collagenation, collagen maturation and scar maturation which are concurrent

though independent.[12] Wound healing progresses through a series of interdependent and overlapping stages in which a variety of re-establishing the integrity of damaged tissue and replacement of lost tissue.[13-14]

Baphia nitida (camwood) is one of the widest spread of the Nigerian Baphias. It is a small tree of the understorey of forests which prefers the wetter areas. It has leaves and branchlets that are glabrous as and habitat in rain forest.[15] *Baphia nitida* leaves are used in folk medicine for the treatment of inflamed and infected umbilical cord in Nigeria. Some various antibiotics are being used for treating a wound and wound infections, but they are more proved to have adverse effects in the human body, and they can equally develop resistance in the presence of the pathological condition. Wound healing could equally occur on its own without medical treatment but there are some factors that associate with a wound such as pains, malaise, the risk of infection and delay healing. If wound healing takes place in a direction away from its normal course, it may result in non-healing, under-healing or overhealing.[16] In this situation, there is a need for an active agent, because there is a probability of secondary infection that may set in due to microbial invasion into the open wound. Therefore, medical attention is required no matter how small the wound. Recently, so much attention has been devoted to exudates of biologically active compounds isolated from plant species used in herbal medicine.[17] Wounds often occur to some people all (not less than 10 % sustains an injury) over the world on a daily basis. Therefore, in order to prevent secondary infection associated with a wound, there is a need to research in the herbal medicine field to produce more available cheap wound healing medication. This research work was to extract, phytochemically screen and evaluate the wound healing property of methanol extract of *Baphia nitida* leaves formulated as an ointment on Wistar rats so as to create more economical and available medication with longer shelf life for wound treatment.

MATERIALS AND METHODS

The fresh leaves of *Baphia nitida* (PCG/UNN/0081) from a family of *Fabaceae* were collected in the month of September, in Nsukka Local Government Area, Enugu State; it was identified and deposited a taxonomist at Department of Pharmacognosy and Environmental medicine, UNN, methanol (Sigma-Aldrich, Germany), Adult Wistar rats obtained from animal house, Department of Pharmacology and Toxicology, University of Nigeria, Nsukka which were treated in accordance with European Community directive for animal experiment (EEC Directive of 1986, 86/609/EEC), distilled water (Lion water, Nsukka, Nigeria), Cicatrin® powder (Glaxo operations, UK), methylated spirit and gentian violet (Kenol Pharm Ltd, Nsukka, All other chemicals, and reagents were of analytical grade.

Preparation of crude extracts

The extraction process adopted the method of Ugwu et al.[18] The fresh leaves were washed with tap water, then cut into smaller fragments and then, sun-dried. The dried leaves were reduced to powder with a hammer mill machine. A 300 g of the milled powder was extracted with 500 ml of methanol using a Soxhlet extractor for 4 h. The mixture was filtered through Whatman No.1 filter paper. The solvent was allowed to evaporate leaving behind dry, deep green solid plant extract which was weighed, stored in a sealed bottle and refrigerated until used. The percentage yield was obtained using equation 1.

$$percentage\ yield\ (\%) = \frac{weight\ of\ the\ yield}{weight\ of\ milled\ extract} \times 100$$

…… Eqn 1

Phytochemical screening

The methanolic extract of *Baphia nitida* was screened for phytochemical components using the methods described by Harborne and Trease.[19, 20] The following plant metabolites were tested: alkaloids, cardiac glycosides, flavonoids, saponins, tannins, and terpenoids.

Test for Alkaloids

A 20 ml quantity of 3 % sulphuric acid in 50 % ethanol was added to 2 g of the extract and heated on a boiling water bath for 10 min., cooled and filtered. A 2 ml of the filtrate was tested with a few drops of Mayer's reagent (Potassium mercuric iodide solution), Dragendorff's reagent (bismuth potassium iodide solution), Wagner's reagent (iodine in potassium iodide solution), and picric acid solution (1 %). The remaining filtrate was placed in 100 ml separating funnel and made alkaline with dilute ammonia solution. The aqueous alkaline solution was separated and extracted with two 5 ml portions of dilute sulphuric acid. The extract was tested with a few drops of Mayer's, Wagner's, Dragendorff's reagents and picric acid solution.

Test for Glycosides

A 5 ml quantity of dilute sulphuric acid was added to 0.1 g of the extract in a test tube and boiled for 15 min on a water bath, then cooled and neutralized with 20 % potassium hydroxide solution. A ten milliliter of a mixture of equal parts of Fehling's solution 1 and 11 were added and boiled further for 5 min.

Test for Saponins

A 20 ml quantity of distilled water was added to 0.25 g of the extract and boiled on a hot water bath for 2 min. The mixture was filtered while hot and allowed to cool and the filtrate was used for the following tests;

The frothing Test

A 5 ml quantity of the filtrate was diluted with 15 ml of distilled water and shaken vigorously.

Emulsion Test

To the frothing solution was added 2 drops of olive oil and the contents shaken vigorously.

Fehlings Test

To 5 ml of the filtrated was added 5 ml of Fehling's solution (equal parts 1 and 11) and the contents were heated on a water bath.

Test for Tannins

A 1 g of the powdered drug extract was boiled with 20 ml of water, filtered and used for the following test.

Ferric Chloride Test

To 3 ml of the filtrate, few drops of ferric chloride were added.

Lead Acetate Test

To a little of the filtrate was added lead acetate solution.

Test for Flavonoids

A 10 ml of ethyl acetate was added to 0.2 g of the extract and heated on a water bath for 3 min. The mixture was cooled, filtered and the filtrate was used for the following tests:

Ammonium Test

A 4 ml of filtrate was shaken with 1 ml of dilute ammonia solution. The layers were allowed to separate.

1% Aluminum Chloride Solution Test

Another 4 ml portion of the filtrate was shaken with 1 ml of 1 % Aluminum Chloride Solution. The layers were allowed to separate.

Test for terpenoids

A 5.0 ml of the extract was mixed with 2.0 ml of chloroform while 3.0 ml of 1.0 M H_2SO4 was carefully added to form a layer. A reddish-brown colouration of the interface was formed which shows the presence of terpenoids.

Animal Design

The animals used in this experiment were cared for and all treatment protocols were performed in accordance with guidelines on animal ethics in Nigeria and the University of Nigeria, Nsukka which complied with European community directive for an animal experiment (EEC Directive of 1986, 86/609/EEC).[21] Twenty healthy Wistar rats of both sexes weighing between 190-210 g were obtained from the animal unit of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka. They were used for screening the wound healing activity of methanol *Baphia nitida* extract. The animals were grouped into five groups of six animals each. The animals were kept for a few weeks to acclimatize before the commencement of the wound healing studies. They have free access to food and clean drinking water throughout the period of the experiment.

Preparation of Ointment

The ointment was prepared according to a standard procedure.[18, 22-24] Different concentrations of the methanol extract (5, 10, and 20 % w/w) and 5 % Cicatrin® powder were added to the ointment base and stirred gently and continuously until a homogeneous product was obtained using a magnetic hot plate. It was allowed to cool to a semi-solid and each of the formulations packed in a clean ointment jar and well labeled.

Evaluation of the extract for wound healing properties

The wound sites were prepared according to Dash *et al.* excision wound model.[25] Each rat was anesthetized with diethyl ether at a dose of 1 ml/kg body weight. The hairs on the one side of the abdomen were clipped off as soon as the drug effect was noticed with a clean sterile surgical blade of size No.20. The clipped area was disinfected with 70 % ethanol. A circular incision of 20 mm in diameter was made on the disinfected area of the skin surface and the skin carefully dissected out. Immediately the wound area was measured by placing a transparent tracing paper over the wound and tracing it out. The tracing paper was placed on a 1 mm graph sheet and traced out. The squares were counted and the area recorded.

The treatment started shortly after creating the wounds by application of 10 g of the various formulated-ointments:

Group 1 received 5 % extract; Group 2 received 10 %, while the Group 3 received 20 % of the medicated ointment. Group 4 received Cicatrin® and served as the positive control, while Group 5 received the ointment base without any drug (placebo) and therefore served as negative control. The drug administration was topically done once daily for 10 days after dressing the wound with methylated spirit. The wound area of each animal was measured under light diethyl ether anesthesia on the 1^{st}, 4^{th}, 7^{th}, 10^{th}, 13^{th}, and 16th-day post-surgery.

Statistical analysis

The percentage of wound healing on the experimental days was carried out using GraphPad InStat Demo (USA). Values are expressed as mean ± SEM (standard error of the mean). Data were calculated and analyzed with a one-way analysis of variance (ANOVA). Differences between means were assessed by a two-tailed student's T-test and $p < 0.05$ was considered statistically significant.

RESULTS

The percentage yield

The percentage yield of the methanol extract was obtained as 12.67 % of 38 g.

Phytochemical screening

The result of the phytochemical test indicated the presence of the following constituents: Alkaloid, flavonoid, cardiac glycosides, terpenoids, tannins, and saponins as shown in Table 1.

Table 1: Phytochemical tests of methanol extract of *Baphia nitida* leaves.

Phytochemicals	Methanolic extract
Flavonoid	++
Glycosides	++
Terpenoids	+
Alkaloids	++
Tannins	++
Saponins	+

[+] low concentration, [++] high concentration

The results of percentage wound healing activity of the animals

The results of percentage wound healing activity of the animals using various concentrations of medicated *Baphia nitida* ointment were shown in Figure 1.

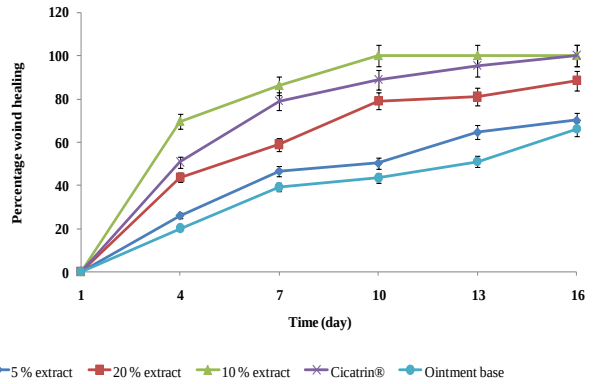

Figure 1: Percentage wound healing activity of *Baphia nitida* leaves on Wistar rats.

DISCUSSION

Phytochemical constituents are attributed to have some important activities such as antioxidant, wound healing activity, antibacterial, anti-inflammatory, etc. An oxidative state has been shown to play a role in the worsening of wound healing. Flavonoids have been established to possess an antioxidant effect.[18, 26-31] In the mechanisms of wound healing, there is stimulation of the production of antioxidants in wound site which provides a favorable environment for tissue healing. Antioxidants may play an important role in the wound healing process and may be a crucial contributory factor in the wound healing property.[18, 32] Antioxidant also improves wound healing and protect tissues from oxidative damage.[33] Flavonoid can salvage for the reactive oxygen species (superoxide anions) and free radicals produced and these reactive intermediates are potentially implicated in delayed wound healing.[34] Therefore, the higher the flavonoids content, the stronger the antioxidant activity.[18, 35] In addition flavonoid and terpenoids also possess both astringent and antimicrobial property which helps in wound contraction and increase the rate of epithelization.[36, 37]

The result showed daily progress in the percentage wound healing activity across the batches throughout the period of the experiment, though at different rates. On the 4th-day post surgery, there was a decrease in wound areas in all the groups even with the ordinary ointment base (placebo and negative control), this means that ointment base has a protective effect over the wound and did not encourage the growth of microorganisms.[22, 37]

The ointment containing 10 % w/w extract had more reduction in the wound area, almost 100 % healing activity within 7th to 10th-day post surgery which was highly significant ($p < 0.05$) to ointment containing 5.0 % and 0.0 % (placebo) extract concentrations. The 10 % percentage wound healing activity was also higher than the standard and the 20 % extract concentration though no significant variations. The wound reduction effect of the 10 % extract ointment showed complete epithelization on the 13th day, while the positive standard showed its own within day 16th. Group 3 (20 % concentration) wound healing was higher than Group 1 (5 % extract concentration) which was higher than Group 5 (placebo) though no significant difference. At higher concentrations of the extract (20 %), there was decreased in diffusion/poor adsorption of the extract into the ointment base that resulted in lower wound healing activity.

CONCLUSION

Methanol *Baphia nitida* leaf extract wound healing activity is as a result of the presence of active phytochemical constituents. *Baphia nitida* is a naturally sourced medicinal plant that contains phytochemicals with antioxidant, astringent, and antimicrobial properties which play important roles in wound healing. In medical practice, any entity whether natural or synthetic to be used as wound healing agent must be able to do so within the first 16 days of the wound.[38] The best wound healing activity of *Baphia nitida* leaf was found at 10 % concentration due to its high wound area reduction that occurred within day 13 post-surgery. This study has justified the folkloric use of *Baphia nitida* leaves in the wound treatment at certain dose concentration. This could be in support by research finding on wound healing activity of *Vernonia amygdalina*.[18, 37] Among all the different extract concentrations, 10 % formulated ointment achieved complete epithelization before 16th day.[36] The best activity was observed in the *Baphia nitida* ointment extract containing 10 % and was taken as the optimum concentration; hence, this could be suggestive that the optimum wound healing activity of *Baphia nitida* could be accepted at 10 % for the methanol leaf extract.

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