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Evaluation of Wound Healing Activity (Excision Wound Model) of Ointment Prepared from Ethanolic Leaves Extract and Fractions of *Cleistopholis staudtii*

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Abstract

Cleistopholis staudtii is a medicinal plant traditionally used in Africa for wound management, but its scientific validation remains limited. This study evaluated the wound-healing efficacy, phytochemical composition, and safety profile of its ethanol extract and solvent fractions. Leaves were macerated in ethanol to obtain a crude extract (19.1% yield), which was fractionated into n-hexane (HF), ethyl acetate (EAF), and n-butanol (BF) fractions, with EAF showing the highest yield (30.4%). Phytochemical analysis revealed abundant flavonoids (8.6%) and phenolics, particularly in EAF and BF. Acute toxicity testing in mice established an LD_{50} exceeding 5000 mg/kg, indicating high safety. In excision wound models (50 rats), topical application of 10% BF and 10% EAF formulations significantly increased wound closure, achieving near-complete healing by Day 18 (comparable to cicatrin powder, $p < 0.05$), while petroleum jelly controls showed minimal improvement. LC-MS analysis identified key bioactive compounds, including kaempferol glycosides (antioxidant), eugenol (antimicrobial), and salicin (anti-inflammatory), which likely synergistically promoted tissue regeneration through anti-inflammatory, antimicrobial, and collagen-modulating mechanisms. These findings scientifically validate the traditional use of *C. staudtii* and highlight its 10% butanol and ethyl acetate fractions as promising candidates for development into standardized wound-healing agents.

Keywords: *Cleistopholis staudtii*; Wound healing; Acute toxicity; Excision**;** Butanol and ethyl acetate fractions

1. Introduction

Our skin is essential to our survival, it senses the environment, maintaining physicochemical and thermal homeostasis, acting as a store of essential nutrients, providing defense, and responding to trauma and injury [1]. A wound is a break in the epithelial integrity and may be accompanied by disruption of the structure and function of underlying normal tissue. It is a clinical entity and is as old as mankind, often considered as major problem in clinical practice [2]. Wound healing is the process that the skin goes through as it repairs damage from wounds after injury that might be caused by physical, chemical, thermal, microbial or immunological insults.

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Many factors can interfere with wound healing process, thus leading to improper or impaired wound healing. These factors may include poor nutrition, insufficient oxygenation, infection, prolonged inflammation, age, diabetes and other diseases, drugs, smoking, alcoholism, depression and others [3]. Impaired wound healing can lead into severe morbidity resulting to long hospitalization of patients. The aim of treating wounds is to shorten the healing time and to reduce risks of undesired complications [4].

Research on wound healing agents is one of the developing areas in modern medical sciences. Many traditional practitioners across the world have valuable information of many plants for treating wounds and burns. The presence of bioactive constituents in plants has urged researchers to screen medicinal plants with a view to determine potential wound healing activities and isolate chemical entities associated with wound healing [5].

This study evaluates the wound healing efficacy of an ointment formulated from the ethanolic leaves extract and fractions of *Cleistopholis staudtii* using an excision wound model in rats. The excisional wounds are one of the most commonly used wound healing models [6] and are considered to resemble acute clinical wounds, which require healing by second intention in humans, that is, the skin edges are not sutured together [7]. This model provides a robust framework for assessing re-epithelialization, granulation tissue formation, and wound contraction [8]. By comparing the effects of crude extract and solvent fractions, this research aims to identify the most therapeutically active components, thereby contributing to the development of evidence-based herbal remedies for wound management.

2. Materials and Method

2.1. Plant material, collection, and authentication

Fresh leaves of *Cleistopholis staudtii* were collected from a single location at Orba, Nsukka, Enugu State, Nigeria on the 4th of May 2021. The plant material was identified and authenticated by Mr. Okeakpu Kyrian of Bioresources Development and Conservation Programme (BDCP).

2.2. Chemicals

The following solvents: ethyl acetate, n-hexane, methanol, and n-butanol were used. All solvents were of analytical grade. Cicatrine powder and ketamine anesthesia both procured from Gacowelt, Pharmaceuticals Ltd. in Awka.

2.3. Animals

Adult Swiss albino mice (25 – 30 g) and rats (180 – 200 g) of both sexes were used for this study. The animals were obtained from the Animal House of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Nigeria. The animals were maintained on standard laboratory animal conditions and fed with rodent feed (Guinea Feeds Nigeria Ltd). They were allowed free access to water ad libitum. All animal experiments were conducted in compliance with NIH guide for care and use of laboratory animals and approved by the Nnamdi Azikiwe University Animal Ethical Committee with approval number NAU/AREC/2025/0076.

2.4. Preparation of plant material

The plant materials were cleaned, spread on a clean sack and placed under the shade for air drying at room temperature for 7 days. The plant materials were further dried in the oven maintained at 40 ^{0}C followed by milling into a coarse powder using modern laboratory electric milling machine. The powdered materials were stored in airtight container before use.

2.5. Plant extraction and fractionation

Two and half kilogram of the pulverized leaves *C. staudtii* extracted by cold maceration in 7.5L of ethanol for 72 h with intermittent shaking. The resulting solution was filtered, and the filtrate concentrated to dryness *in vacuo* using rotary evaporator (RE300 Model, United Kingdom) at 40°C. Then 200 g of the extract was dissolved in 400 ml of water and subjected to liquid-liquid partition successively and exhaustively with n-hexane, ethyl acetate and n-butanol in increasing order of polarity. Fractions soluble in these solvents were concentrated *in vacuo* using rotary evaporator to obtain the n-hexane fraction (HF), ethyl acetate fraction (EAF) and n-butanol fraction (BF). The remaining fraction was the water fraction (WF). The plant extract and fractions were stored in refrigerator between 0-4°C.

2.6. Acute Toxicity (LD50)

The acute toxicity of the extract was determined in mice using the method of [9]. The tests were done in phases. The first phase (determination of the toxic range) involves 3 groups of 3 mice that received 10, 100, and 1000 mg/kg orally. The treated mice were observed for 24 h for mortality. The death pattern in the first phase determined the doses used for the second phase. Because there were no deaths recorded in the first phase, a fresh batch of four mice received 1000, 1600, 2900, and 5000 mg/kg of the extract each. The treated animals were observed for lethality or signs of acute intoxication for 24 h. The LD50 was estimated as the geometric mean of the highest nonlethal dose and the least toxic dose.

2.7. Preparation of Extract Ointment for Wound Healing Activity

Preparation of ointment Simple ointment BP was formulated using formula as shown in Table 1 below. Using fusion method, hard paraffin, soft paraffin, cetostearyl alcohol and wool fat were melted successively in decreasing order of melting point and the fluid mixture stirred continuously until cooled, avoiding aeration [10]. Ointment of *C. staudtii* ethanolic extract and fractions were prepared by melting 190 mg for 5% and 180mg for 10% of simple ointment BP in a beaker over a hot water bath maintained at 80 ^{o}C, then 40 ml of molten ointment was taken and emptied into a beaker and 10 mg (for 5% w/w preparation) and 20 mg (for 10% w/w preparation) of *C. staudtii* ethanolic extract was added and mixed vigorously. The mixture was transferred into an ointment jar and stored.

Table 1 Formula for the preparation of Simple ointment

Ingredients	BP Formulae (g)	Amount used (g)	Melting point (ºC)
Wool fat	50	12.5	36-44
Hard paraffin	50	12.5	48-66
Cetostearyl alcohol	50	12.5	50
White soft paraffin	850	212.5	38-56
Total	1000	250	

2.8. The Wound Healing Activity of The *Cleistopholis staudtii* Extracts.

Wound healing effect of *Cleistopholis staudtii* was carried out using animal wound excision as described by Rashed [11] with slight modification. A total of 50 adult albino rats both sexes were used. They were grouped into 10 groups of 5 per group. The animals were anesthetized with 10mg/kg ketamine i.p prior to wound creation. The furs at the back of the animal were shaved. Excision of wound uniform of 1.5cm diameter circular was created along marking using toothed forceps and pointed scissors. The wound was left untreated for 18hours for complete wound expansion, then, the groups received treatment. Group 1 received petroleum jelly serve as a control; Group 2 received cicatrin powder; Group 3 received 5% extract of the crude extract, Group 4 received 10% extract of the crude extract; Group 5 received 5% n-hexane fraction; Group 6 received 10% n-hexane fraction; Group 7: received 5% Ethyl acetate fraction; Group 8 received 10% ethyl acetate fraction; Group 9 received 5% butanol fraction; Group 10 received 10% butanol fraction. The drug was applied once daily until complete epithelization. The measurement of the wound areas of the excision wound model was taken on 0, 3rd, 6th, 9th, 12th, 15th, and 18th days. The wound was measure at 3 days interval using meter rule. The degree of wound heal was calculated using the formula:

$$(A\text{-}B)/A \times 100$$

A= mean wound size at day 0

B= mean wound size of corresponding days.

2.9. Statistical analysis

Results were presented as mean ± Standard error of mean (SEM). The analysis of variance in the outcome of the treatment (one way ANOVA) was done using Statistical Package for Social Science (SPSS, version 20). Multiple comparisons for post hoc analysis was done using Turkey's test. The differences between the treatment groups were determined by multiple comparisons of mean ranks for all groups. In all cases, a probability error of less than 0.05 was

selected as the criterion for statistical significance. Calculation of fifty percent inhibitory concentration (IC50) of the extracts and fractions was carried out using regression equation in Microsoft Excel, 2007.

3. Result

Table 2 Result of acute toxicity studies

PHASE 1	DOSE (ml/kg)	NUMBER OF DEATH
Group 1	10	0/3
Group 2	100	0/3
Group 3	1000	0/3
PHASE 2	DOSE (ml/kg)	NUMBER OF DEATH
Group 1	1600	0/1
Group 2	2900	0/1
Group 3	5000	0/1

The oral LD50 was therefore estimated to be above 5000mg/kg in mice

Table 3 Percentage wound contraction

DAYS	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18
Petroleum Jelly	-11.94	-16.42	-14.93	-11.94	-7.46	0.00
Cicatrin Powder	6.49	25.97	31.17	44.16	80.52	100.00
5% Extract	2.60	5.19	12.99	31.17	63.64	87.01
10% Extract	12.99	24.68	31.17	44.16	77.92	100.00
5% N-Hexane Fraction	5.19	11.69	18.18	28.57	31.17	61.04
10% N-Hexane Fraction	1.37	4.11	15.07	20.55	35.62	69.86
5% E.A Fraction	4.11	13.70	15.07	28.77	49.32	75.34
10% E.A F	6.85	10.96	15.07	35.62	56.16	86.30
5% Butanol Fraction	6.49	12.99	24.68	31.17	44.16	80.52
10% Butanol Fraction	11.69	18.18	28.57	44.16	87.01	87.01

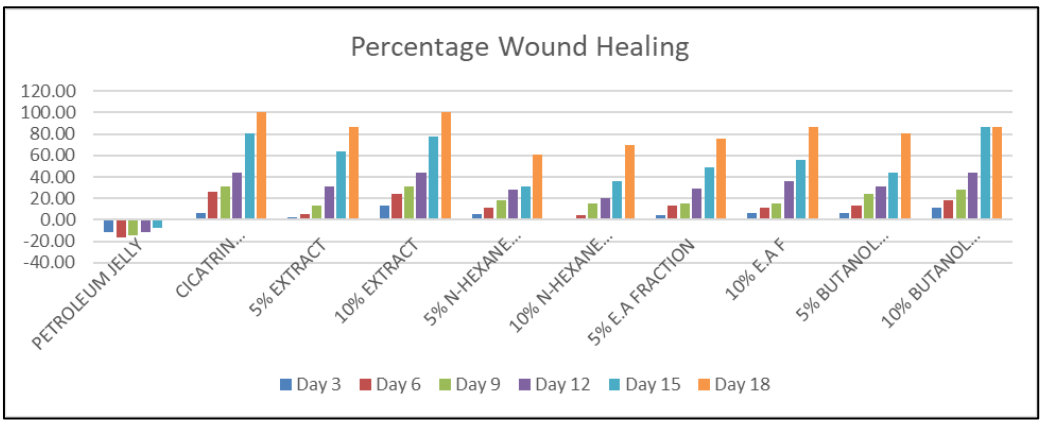

Figure 1 A bar chart of percentage wound healing against time

4. Discussion

The acute toxicity study conducted in two phases revealed no mortality in mice at doses up to 5000 mg/kg of the extract, indicating a high margin of safety. According to the OECD guideline 425 for acute oral toxicity, substances with LD_{50} values greater than 5000 mg/kg are considered relatively non-toxic. This suggests that the ethanol extract of the plant under investigation poses minimal acute toxicological risk when administered orally, at least within the tested dose range. The absence of signs of toxicity or death further supports its safety for potential therapeutic use, including topical applications [12].

The wound healing data presented in Table 3 demonstrated the ability of the extract and its various fractions to promote wound shrinkage over an 18-day period in a dose-dependent manner. Among all treatment groups, the 10% crude extract and the 10% butanol fraction exhibited the most significant reduction in wound size, achieving near-complete or complete wound closure by Day 18, comparable to the standard treatment, Cicatrin powder. The butanol fraction (10%) in particular showed rapid and consistent wound contraction, suggesting it may contain a higher concentration of bioactive polar constituents such as flavonoids, tannins, or saponins, compounds known to enhance wound healing by enhancing fibroblast proliferation, collagen synthesis, angiogenesis, and anti-inflammatory effects [13, 14]. The ethyl acetate fractions (5% and 10%) also showed good wound healing activity, though slightly less effective than the butanol fraction. This indicates that semi-polar compounds may also contribute significantly to the healing process. In contrast, the n-hexane fractions, which are rich in non-polar constituents like lipids and essential oils, showed the least wound contraction among the extract groups, although still better than the negative control (petroleum jelly), which showed negligible healing over the 18-day period. This confirms that petroleum jelly, while occlusive, lacks intrinsic wound healing properties.

These findings suggest that the wound healing activity of the plant is attributable to the presence of bioactive polar compounds that are more concentrated in the butanol and ethyl acetate fractions. The dose-dependent responses further support the efficacy of the extract and validate the traditional use of this plant in the management of wounds and skin injuries.

5. Conclusion

The ethanol extract and its fractions, particularly the 10% butanol fraction, showed potent wound healing activity that is comparable to standard treatment, with no observed acute toxicity at doses up to 5000 mg/kg. These findings underscore the potential of the plant as a safe and effective source of wound healing agents, meriting further phytochemical investigation and mechanism-based studies.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare the absence of any conflicts of interest

Statement of ethical approval

Ethical approval for the use of Animals for the study was issued by Nnamdi Azikiwe University Animal Ethical Committee with approval number NAU/AREC/2025/0076.

References

[1] Shedoeva, A., Leavesley, D., Upton, Z., & Fan, C. (2019). Wound healing and the use of medicinal plants. Evidence-Based Complementary and Alternative

[2] Kokane D.D., More R.Y., Kale M.B., Nehete M.N., Mehendale P.C, Gadgoli C.H. Evaluation of wound healing activity of root of *Mimosa pudica*. Journal of Ethnopharmacology. 2009; 124 (2):311-315

[3] Nagori, B. P., & Solanki, R. (2011). Role of medicinal plants in wound healing. Research Journal of Medicinal Plant, 5(4), 392-405.

[4] MacKay, D., & Miller, A. L. (2003). Nutritional support for wound healing. Alternative medicine review, 8(4).

[5] Kumar G S, Jayaveera K N, Kumar C K A, Sanjay U P, Swamy B M V, Kumar D V K. Antimicrobial effects of Indian medicinal plants against acne-inducing bacteria. Trop J Pharm Res. 2007; 6: 717–723.

[6] Nauta AC, Gurtner GC, Longaker MT. Adult stem cells in small animal wound healing models In: Gourdie RG, Myers TA, eds. Wound Regeneration and Repair Methods and Protocols. New York, NY: Humana Press; 2013:81-98.

[7] Masson-Meyers DS, Andrade TAM, Caetano GF, Guimaraes FR, Leite MN, Leite SN, Frade MAC. Experimental models and methods for cutaneous wound healing assessment. Internationa Journal of Experimantal Pathology. 2020; 101(1-2):21-37.

[8] Morton JJ and Malone MH. Evaluation of vulneray activity by an open wound procedure in rats. Archives of International Pharmacodynamics and Therapy. 1972; 196 (1):117-26.

[9] Lorke, D. (1983). A new approach to practical acute toxicity testing. Archives of Toxicology, 54(4), 275–287.

[10] Chukwunonso Chukwudike Onwuzuligbo, Ifesinachi Sunday Okafor and Amarachukwu Ukamaka Onwuzuligbo. Formulation and evaluation of antimicrobial properties of ointment of Chromolaena odorata ethanolic extract. World Journal of Biology Pharmacy and Health Sciences, 2025, 22(01), 028-036.

[11] Rashed AN, Afifi FU, Disi AM. Simple evaluation of the wound healing activity of a crude extract of Portulaca oleracea L. (growing in Jordan) in Mus musculus JVI-1. Journal of Ethnopharmacology. 2003; 88(2-3):131-6

[12] Organization for Economic Cooperation and Development (2008) Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure, OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing, Paris.

[13] Fahimi S, Abdollahi M, Mortazavi SA, Hajimehdipoor H, Abdolghaffari AH, Rezvanfar MA. Wound healing activity of a traditionally used poly herbal product in a burn wound model in rats. Iran Red Crescent Med J. 2015; 17(9).

[14] Mutuma G.G., Joseph N, Alex M, Silas K. Phytochemical and anti-inflammatory analysis of Prunus africana bark extract. Res J Pharm. 2020; 7:31–38.