WOUND HEALING ACTIVITY OF ETHANOL LEAF EXTRACT OF *ERYTHRINA SENEGALENSIS*

Ilodigwe, E. E. [1], Okonkwo, B. E. [2], Agbata C. A.[3], Ajaghaku, D. L. [4], and Eze, P. M. [5*]

[1]Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka-Nigeria.

[2]Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka-Nigeria.

[3]Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka-Nigeria.

[4]Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka-Nigeria.

[5]Department of Pharmacetical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka-Nigeria.

ABSTRACT

Aim: This study investigates the wound healing activity of ethanol leaf extract of *Erythrina senegalensis* using excision wound model on albino rats.

Methodology: Several herbal extract formulations were prepared with Petroleum Jelly ointment base. Cicatrin® powder (neomycin-bacitracin) was used as the positive control. The various ointment formulations were applied topically on the wounds daily for 21 days. Daily wound contraction and epithelialisation times were recorded for each group. The antibacterial activity of the extract was also evaluated against some bacteria species implicated in wound infections. The following test organisms were used: *Staphylococcus aureus, Pseudomonas aeruginosa, klebsiella pneumoniae* and *Escherichia coli.*

Results: The Phytochemical analysis revealed that alkaloids were abundant in the extract. The herbal ointment at various concentrations showed significant (P<.05) increase in percentage wound contraction on day 9 – 21 compared with the control group that received only the ointment base. The contraction produced by 40% w/w of the extract was similar to that of Cicatrin® powder on day 6 – 21. The results also revealed significant (P<.05) reduction in epithelialisation time exhibited by the extract treated animals compared to those of the control group. The result of antimicrobial studies showed that the extract inhibited the test organisms at concentrations ranging from 200 to 12.5 mg/mL. The Minimum Inhibitory Concentrations (MICs) of the extract on the test isolates was recorded at 25mg/mL for both *S. aureus* and *E. coli,* and 6.25mg/ml for *K. pneumoniae*. *P. aeruginosa* showed no susceptibility to both the extract and the control drug at the concentrations evaluated.

Conclusion: The marked reduction of wound size and epithelialisation time by the extract is an indication of its wound healing potentials. Also, the antibacterial activity of this plant against bacterial species implicated in wound infections may contribute to the enhanced wound healing activity.

Keywords: *Erythrina senegalensis,* wound healing, antibacterial activity, epithelialisation, wound contraction.

*Tel.: +2348063809147
E-mail address: ezep2004@hotmail.com

INTRODUCTION

Wounds arise due to physical, chemical, immunological or microbial insult to the tissue [1]. The healing process is usually complex, dynamic and varies among different tissue types. However, homeostasis, inflammatory reaction, proliferation and remodelling are the common similarities shared in wound healing processes [2]. These healing processes are geared towards restoration of integrity of the damaged tissue [3]. Traditionally, wounds have been treated mostly topically with different medicinal plants or with their extract solely or in combination with some other plant parts. Approximately, one-third of all traditional medicines in use are for the treatment of wounds and skin disorders compared to only 1-3 % of modern drugs [4].

Several researches have been carried out in the area of wound treatment and management especially through medicinal plants. Wound healing activities of some plants have been reported, these include: *Alternanthera sessilis* [5], *Lycopodium serratum* [6], *Morinda citrifolia* [7], *Sesamum indicum* [8], *Napoleona imperialis* [9], *Lawsonia alba* [10].

Erythrina senegalensis is commonly found in Mali, Senegal and Nigeria. It is mainly grown in West Africa as an ornamental plant [11]. The leaves are used to treat malaria, gastrointestinal disorders, fever, diarrhea, jaundice and pain [12]. The stem barks have been shown to possess antimicrobial activities [13] and also inhibits HIV-1 protease [14]. The Hepatoprotective activities of the stem bark have also been documented [15]. The bark and root decoction is used for stomach disorder and wounds [11]. In Cameroon and Nigeria, decoctions made from different parts of the plant are taken orally or in body baths to treat malaria, fever, cough, snake bites, inflammation and prostate [12]. In Cameroon also, the stem bark is used to treat liver disorders [16]. Prynylflavonoids; 8-prenylleutone; auriculatin; erysengalensin O, D and N; derrone; alpinumisoflavone; and 6, 8-diprenylgenisten have all been isolated from *E. senegalensis* and have demonstrated antimicrobial and pharmacological activities [17].

The leaf of *E. senegalensis* is very popular in wound management in South Eastern Nigeria because it offers inexpensive, readily available and effective approach to treatment of wounds, yet scientific validation of this pharmacological activity has not been done. This study evaluates the wound healing activity of ethanol leaf extract of *E. senegalensis* using Swiss albino rats.

MATERIALS AND METHODS

MATERIALS

Plant Material

The fresh leaves of *E. senegalensis* were collected in May, 2013 from young matured plants in Agulu, Anambra State, Nigeria and was identified and authenticated by a taxonomist, Mr. Paulinus Ugwuozor, of the Department of Botany, Nnamdi Azikiwe University, Awka-Nigeria. Plant authentication voucher specimen number: BDCP 709. The leaves were air dried under room temperature for seven days and pulverized.

Animals

Thirty (30) healthy and wound-free four (4) months old Swiss albino rats of both sexes, weighing 200-230g, were obtained from animal house of the Department of Pharmacology & Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka-Nigeria. The animals were allowed free access to food and water *ad libitum* and were placed under standard Laboratory animal house condition.

Test Organisms

Pure cultures of *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa, and Klebsiella pneumoniae* obtained from the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka-Nigeria were used in this study.

Culture Media and Reagents

Culture media used were Nutrient Agar, Nutrient Broth and Mueller-Hinton Agar (Oxoid Limited, England). Reagents used include McFarland 0·5 turbidity standard (prepared from barium chloride sulfuric acid and water), Ethanol (SIGMA-ALDRICH Inc., Germany), sodium chloride (BDH Chemicals, England), Cicatrin® (neomycin-bacitracin), distilled water, dimethyl sulfoxide (DMSO), etc.

METHODS

Plant Extraction

The dried powdered plant material weighing 555 g was cold macerated using 2 L of 70% v/v ethanol for 48 hours with intermittent agitation. The extract was filtered using Whatman No.1 filter paper, and the filtrates obtained were concentrated to dryness at 40^0C in vacuum using rotary evaporator.

Phytochemical Test

The qualitative phytochemical analysis of *Erythrina senegalensis* extracts was carried out using standard protocols [18].

Antimicrobial Activity

The antibacterial activity of the ethanol extract of *E. senegalensis* was evaluated against bacteria species implicated in wound infections. The following test organisms were used: *Staphylococcus aureus, Pseudomonas aeruginosa, klebsiella pneumoniae* and *Escherichia coli. Preliminary antibacterial* screening of the extract on test organisms was carried out by the Agar well diffusion method and the Minimum Inhibitory Concentrations (MICs) were determined by the Agar dilution method.

Primary Screening of Extracts for Antibacterial Activity

The antibacterial activity of the extract was determined by the agar well diffusion method. Dilutions of 100.00, 50.00, 25.00, 12.50, 6.25 and 3.13 mg/ml were prepared in a 2-fold serial dilution from a stock solution of 200mg/mL. Twenty (20) mL of molten MH agar was poured into sterile Petri dishes (90 mm) and allowed to set. Standardized concentrations (McFarland 0.5) of overnight cultures of test isolates were swabbed aseptically on the agar plates and holes of 6 mm diameter bored using sterile metal cork-borer. 20µl of the various dilutions of each extract and control were put in each hole under aseptic condition, kept at room temperature for 1 hour to allow the agents to diffuse into the agar medium and incubated accordingly. Cicatrin® (25µg/mL) was used as positive control, while DMSO was used as the negative control. The plates were then incubated at 37^oC for 24 hours and the inhibition zones diameters (IZDs) were measured and recorded. The size of the cork borer (6mm) was deducted from the values recorded for the IZDs to get the actual diameter. This procedure was conducted in duplicate and the mean IZDs calculated and recorded.

Determination of Minimum Inhibitory Concentration (MIC) of the Extracts on Test Isolates

Minimum inhibitory concentration (MIC) is defined as the lowest concentration of the antimicrobial agent that inhibits the bacterial growth. The MICs of the plant extract on the test isolates were determined by the Agar dilution method. A stock solution (1000mg/ml) of the plant extract was diluted in a 2-fold serial dilution to obtain the following concentrations: 500.00, 250.00, 125.00, 62.50, and 31.25 mg/mL. Agar plates were prepared by pouring 19mL of MH agar into sterile Petri plates containing 1mL of the various dilutions of the extract to obtain final plate concentrations of 25.00, 12.50, 6.25, 3.13 and 1.56 mg/mL. The test isolates were grown for 18 hours in Nutrient broth and culture suspensions adjusted to McFarland 0.5 were streaked onto the surface of the agar plates containing dilutions of the extract. Plates were incubated at 37^oC for 24 hours, after which all plates were observed for growth. The minimum concentration of the extracts completely inhibiting the growth of each organism was taken as the MIC.

Formulation of Herbal Ointment

The herbal extract formulation was prepared in the form of a simple ointment for topical application. The ointment base (10 g Petroleum jelly B.P) was used, and 1.0, 2.0 and 4.0 g of the extract were incorporated into the base using the trituration method of preparing medicated ointments. The blank control ointment was prepared with only the ointment base without the extract. For each batch, 10 g of blank petroleum jelly B.P. was weighed and melted in a water bath at 70^oC. The required quantities of the extract was weighed, added to the molten ointment base at 40°C, mixed and swirled gently and continuously until a homogenous dispersion was obtained.

Wound Induction and Treatment Using Excision Wound Model

Thirty (30) Swiss albino rats were randomly divided into 5 groups of six animals each. They were anesthetized with chloroform by open mask method and excision wounds inflicted [19]. The thickness of the excision wound of circular area was $177mm^2$ and 2 mm depth created along the *latissimus dorsi* *(dorsal thoracic region)*. Haemostasis was achieved by blotting the wound with cotton swab soaked in normal saline. The wounds on each animals were treated topically daily with the prepared formulations, vehicle (Petroleum jelly B.P) and Cicatrin® powder, by applying appropriate quantity of these drugs that sufficiently covers the wound area.

The wound area was measured every 3 days and the percentage wound contraction estimated, until complete epithelialization occurred. The wound contraction was calculated as; % reduction in wound area with respect to initial wound area.

$$\% \text{ contraction} = (W_{AO} - W_{AT}) \text{ X } 100$$

W_{AO} = % wound contraction on day 1

W_{AT} = % wound contraction on day T (i.e. day 2-20)

STATISTICAL ANALYSES

Results were expressed as mean ± standard error of mean (SEM). Data obtained from the percentage wound contraction were subjected to the one way ANOVA using SPSS 17.0 and Student t-test. ($P<.05$) was considered to be significant.

RESULTS AND DISCUSSION

Table 1: Phytochemical Analysis of *E. senegalensis*

Phytoconstituents	Occurrence
Alkaloids	+++
Carbohydrates	+
Proteins	-
Saponins	-
Tannins	+
Flavonoids	-
Glysosides	++

+ = present in trace amount, ++ = present in appreciable amount
+++ = Abundant, - = Absent

Table 2: Inhibition Zone Diameters (mm) of *E. senegalensis* against Test Bacteria

Test Isolates	Concentration (mg/mL)								
								Controls	
	200.00	**100.00**	**50.00**	**25.00**	**12.50**	**6.25**	**3.13**	**Positive Cicatrin® (25mg/ml)**	**Negative DMSO**
E. coli	5±0.5	4±0.5	2±0.0	0±0.0	0±0.0	0±0.0	0±0.0	7±0.0	0±0.0
S. aureus	6±0.0	4±0.0	2±0.0	0±0.0	0±0.0	0±0.0	0±0.0	5±0.5	0±0.0
P. aeruginosa	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
K. pneumoniae	8±0.0	6±0.0	4±0.0	3±0.5	2±0.0	0±0.0	0±0.0	8±0.0	0±0.0

Table 3: MICs of *E. senegalensis* Against Test Isolates

Test Organisms	MICs (mg/mL)	
	E. senegalensis	**Cicatrin®**
E. coli	25.00	3.13
S. aureus	25.00	3.13
P. aeruginosa	-	50.00
K. pneumoniae	6.25	1.56

Table 4: Percentage Wound Contraction of Ethanol Extract of *Erythrina senegalensis*

Treatment Groups	% Wound Contraction ± SEM						
	Day 3	**Day 6**	**Day 9**	**Day 12**	**Day 15**	**Day 18**	**Day 21**
10% w/w Extract Ointment	22.6±4.1A	49.4± 6.7A	70.4±3.7B*	77.4±1.8 AB	87.0±3.4A	96.0± 0.5B*	99.6±0.4B*
20% w/w Extract Ointment	21.8±3.4A	50.8±4.4A	70.4± 4.1B*	81.4±2.8B*	94.20±0.73*	98.6±6.5 C*	100.0±0.0B*
40%w/w Extract Ointment	19.0±2.7A	57.2±3.3AB *	73.2±3.0B*	85.4±2.1B*	94.8±1.5B*	99.8±0.20C*	100.0±0.0B*
Blank Ointment (Negative control)	21.4±3.6A	41.8±5.3A	55.2±3.8A	70.4±3.7A*	83.2±3.0A	92.6±1.0A	97.2±0.4A

Number per group = 5
The mean ± SD in the same column for the same category of percentage wound contraction followed by same letter do not differ significantly at P < 0.05 (student Newman Keuls test).
* P<0.05 compared with negative control.

Table 5: Epithelialisation Time of Different Formulated Ointments of *E. senegalensis*

Treatment Groups	Mean Epithelialization time (days)±SD
10% w/w Extract Ointment	16.0±1.0*
20%w/w Extract Ointment	15.6±1.1*
40% w/w Extract Ointment	14.8±0.8*
Blank Ointment (Negative control)	22.6±1.5
Cicatrin® (Standard)	14.4±0.5*

* P<.05 compared with negative control.

The Phytochemical analysis revealed that alkaloids were abundant in the extract (Table 1). Alkaloids have been documented by many studies to possess wound healing activity [20,21]. The abundance of this phytocompound may have contributed to the wound healing activity exhibited by the extract.

Wound healing has been documented to be delayed or impaired by both systemic and local factors [22]. One of the important local factors that may delay or prevent speedy resolution of wound is bacterial infections. It usually prolongs the inflammatory phase of wound healing [23]. Also wounds are known to be easy portal for infections as they provide suitable medium for the proliferation of microbial organisms [24]. The result of antimicrobial studies showed that the extract inhibited the test organisms at concentrations ranging from 12.5 to 200 mg/ml (Table 2), although the positive control drug, Cicatrin® (neomycin-bacitracin) recorded higher activities at a concentration of 25 mg/ml. Table 3 shows the MICs of both the extract and the control drug on the test isolates. The MICs of the extract was recorded at 25 mg/ml for both *S. aureus* and *E. coli,* and 6.25mg/ml for *K. pneumoniae*. *P. aeruginosa* showed no susceptibility to both the extract and the control drug at the concentrations evaluated. This may be due to the intrinsically resistant nature of the organism. *P. aeruginosa, S. aureus, E. coli and K. pneumoniae* are common micro-organisms seen in infected wounds [25,26]. The antibacterial activity of this plant against these bacterial species implicated in wound infections may have contributed to its enhanced wound healing activity.

Wound contraction is a ubiquitous feature of excision wounds and together with new extra cellular matrix formation and epidermal regeneration, affects full wound closure [27]. The extract at various concentrations showed significant (P<.05) increase in percentage wound contraction on days 9 – 21 compared with the control group that received only the ointment base (Table 4). The contraction produced by 40% w/w of the extract was similar to that of Cicatrin® powder on days 6 – 21. The process of wound contraction have been documented as useful mechanism for rapid minimization of exposure of underlying tissue to hazardous external environmental factors that may delay the healing process [28]. The markedly reduction of wound size by the extract is an indication of its wound healing potentials.

Following excision wounds, reconstruction of injured epithelium is crucial for re-establishment of the barrier function of the skin [29]. The significant (P<.05) reduction in epithelialisation time compared with the control (Table 5) exhibited by the extract treated animals is another indication that the extract accelerated the complete wound healing processes.

CONCLUSION

The results of this study justify the traditional use of this plant in the management of superficial wounds.

COMPETING INTERESTS

The authors declare no competing of interests.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) and all experiments have been examined and approved by the appropriate ethics committee. Ethical committee approval number: EC/NAU/PHARM/013/15.

REFERENCES

1. Rajinder PS, Verma PK, Pankaj NK. Medicinal plants and their role in wound healing. Vetscan. 2008;3(1):1-7.

2. LiJuan GU, Eunkyong MO, Zhittong Yang, ZheMing F, Baishen S, Chun YW, XueMei Z, JianFeng B, Changkeun S. Effects of red deer antlers on cutaneous wound healing in full thickness rat models. Asian-Aust. J. Anim. Sci. 2008; 21(2):277-290.

3. Barua CC, Talukdar A, Begum SA, Sarma DK, Fathak DC, Barua AG, Bora RS. Wound healing activities of methanolic extract of leaves of *Alternanthera brasiliana* Kuntz using in vivo and invitro models. Ind. Exp. Biol. 2009; 47:1001-1005.

4. Mantle D, Gok MA, Lennard TWJ. Adverse and beneficial effect of plant extracts on skin and skin disorders: Adverse drug reaction. Toxicol Rev. 2001; 20(2):89-103.

5. Jalalpure SS, Agrawal N, Patil MB, Chimkode R, Tripathi A. Antimicrobial and wound healing activities of leaves of *Alternanthera sessilis*. Int. J. Green Pharm. 2008; 2:141-144.

6. Manjunatha BK, Krishna V, Vidya SM, Mankani KL, Manohara YN. Wound healing activity of *Lycopodium serratum*. Ind. J. Pharm. Sci. 2007; 69:283-287.

7. Nayak BS, Sandiford S, Maxwell A. Evaluation of the wound-healing activity of ethanolic extract of *Morinda citrifola* leaf. Evidence-based complementary and alternative medicine. 2007; 7:1093-1095.

8. Dhumal SV, Kulkarni SR. Antibacterial and wound healing activity of roots of *Sesamum indicum*. Indian Drugs. 2007; 44:937-944.

9. Esimone CO, Ibezim EC, Chah KF. The wound healing effect of herbal oinments formulated with *Napoleona imperialis*. J. Pharm. Allied Sci. 2005; 3:294-299.

10. Mandawade SD, Patil KS. Wound healing potential of some active principles of *Lawsonia alba* leaves. Ind. J. Pharm. Sci. 2003; 65:390-394.

11. Tepongning RN, Yerbanga SR, Dori GU, Lucantoni L, Lupidi G, Habluetzel A. In vivo efficacy and toxicity studies on *Erythrina senegalensis* and Khaya ivorensis used as herbal remedies for malaria prevention in Cameroon. European J of Medicinal Plants. 2013; 3(3):454-464.

12. Togola A, Austarheim I, Theis A, Diallo DH, Paulsen BS. Ethnopharmacological uses of *Erythrina senegalensis*: a comparison of three areas in Mali, and a link between traditional knowledge and modern biological science. Journal of ethnobiology and ethnomedicine. 2008; 4:6.

13. Doughari H. Evaluation of antimicrobial potentials of stem bark extract of *Erythrina senegalensis* DC. Afr. J. Microbiol. Res. 2010; 4(17):1836-1841.

14. Lee J, Oh WK, Ahn JS, Kim YH, Mbafor JT, Wandji J and Fomun ZT. Prenylisoflavonoids from *Erythrina senegalensis* as novel HIV-1 protease inhibitors. Planta Med. 2009; 75:268-270.

15. Donfack JH, Njayou FN, Ngameni B, Tchana A, Chuisseu PD, Finzi PV. In vitro Hepatoprotective and antioxidant activities of diprenylated isoflavonoids from *Erythrina senegalensis* (fabaceae). Asian Journal of Traditional Medicines. 2008; 3(5):172-178.

16. Atsamo AD, Nguelefack TB, Datte JY, Kamanyi A. Acute and suchronic oral toxicity assessment of the aqueous extract from the stem bark of *Erythrina Senegalensis* DC (fabaceae) in rodents. J Ethnopharmacol. 2011; 134(3):697-702.

17. Otimenym SO, Uzochukwu DC. Spasmolytic and Anti-Diarrhea effect of the bark of *Erythrina senegalensis* and root of Kigalia Africana. Asian Journal of Pharmaceutical and Clinical Research. 2013; 3(4):11-14.

18. Trease GE, Evans WC. Textbook of pharmacognosy 12th ed. Bailliere Tindal, London. 1983; 343-383.

19. Thakur R,Jain N,Pathak R, Sandhu S.Practice in wound Healing of studies of plants. Evidence-based complementary and alternative medicine. 2011; 17.

20. Zyuz'kov GN, Krapivin AV, Nesterova YU, Povetieva TN, Zhdanov VV, Suslov NI, *et al.* Mechanisms of regeneratory effects of Baikal aconite diterpene alkaloids. Bulletin of Experimental Biology and Medicine. 2012; 153(6):847-851.

21. Nagappan T, Segaran TC, Wahid MEA, Ramasamy P, Vairappan CS. Efficiency of carbazole alkaloids, essential oil and extract of murrayan koenigii in enhancing subcutaneous wound healing in rats. Molecules. 2012; 17:14449-14463.

22. Molan H. Textbook of Pathology. 5th ed. Japee Brothers Medical Publisher, India; 2005.

23. Menke NB, Witten DG, Bonchev DG, Diegelmann RF. Impaired wound healing. Clinical Dermatological Journal. 2002; 25:19-25.

24. Odimegwu D, Ibezim E., Esimone C, Nworu C, Okoye F. Wound healing and antibacterial activities of the extract of *Dissotis theifolia* stem formulated in a simple ointment base: Journal of Medicinal Plants Research. 2008; 2:11-16.

25. Kumar V, Abbas KA, Fausto N, and Mitchell RN. Robbins Basic Pathology. 8th ed. Sauders, Pennsylannvia; 2007:68-78.

26. Khoo Y, Halim AS, Singh KB, Mohamed N. Wound contraction effects and antimicrobial properties of Tualang honey on full-thickness burn wounds in rats in comparison to hydrofibre. BMC Complementary and Alternative Medicine. 2010; 10(48):1-8.

27. Olsen L, Sherratt JA, Maini PK. A mechanochemical model for adult dermal wound contraction. Journal of Biological System. 1995; 3(4):1021-1031.

28. Fred V, Olmer VR. A mathematical model for wound contraction and angiogenesis, tissue regeneration- from basic biology to clinical application. Jamie D, editor. In Tech; 2012.

29. Monaco JL, Lawrence WT. Acute wound healing: An overview. Clin Plastic Surg 2003; 30:1-12.