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<0.05) 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<0.05) 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], Napoleon 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]. Prynyllflavonoids; 8-prenyleutone; auriculatin; erylengalenin O, D and N; derrone; alpinumisoflavone; and 6, 8-diprenylgenistin 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°C 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 200µg/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°C 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°C 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°C. 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 177mm² 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.

\[
\% \text{ contraction} = (W_{AO} - W_{AT}) \times 100
\]

\[
W_{AO} = \% \text{ wound contraction on day 1}
\]

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

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

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+++</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>++</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Test Isolates</th>
<th>Concentration (mg/mL)</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200.00</td>
<td>100.00</td>
</tr>
<tr>
<td>E. coli</td>
<td>5±0.5</td>
<td>4±0.5</td>
</tr>
<tr>
<td>S. aureus</td>
<td>6±0.0</td>
<td>4±0.0</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>0±0.0</td>
<td>0±0.0</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>8±0.0</td>
<td>6±0.0</td>
</tr>
</tbody>
</table>
Table 3: MICs of *E. senegalensis* Against Test Isolates

<table>
<thead>
<tr>
<th>Test Organisms</th>
<th>Mic (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>25.00</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>25.00</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>6.25</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>6.25</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>% Wound Contraction ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 3</td>
</tr>
<tr>
<td>10% w/w Extract Ointment</td>
<td>22.6±4.1A</td>
</tr>
<tr>
<td>20% w/w Extract Ointment</td>
<td>21.8±3.4A</td>
</tr>
<tr>
<td>40% w/w Extract Ointment</td>
<td>19.0±2.7A</td>
</tr>
<tr>
<td>Blank Ointment (Negative control)</td>
<td>21.4±3.6A</td>
</tr>
</tbody>
</table>

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

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

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**


