EXCISION AND INCISION WOUND HEALING POTENTIAL OF SABA FLORIDA (Benth) LEAF EXTRACT IN RATTUS NOVERGICUS

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ABSTRACT

Saba florida (Benth) belongs to the family Apocynaceae. It is very abundant in undisturbed forest and coastal regions in Africa. It is a folkloric medicine for a variety of ailments including skin ulcer. However there are no scientific reports on wound healing activity of the plant Saba florida (Benth). This study was carried out to evaluate the wound healing potential of Saba florida leaf extract in Rattus novergicus as experimental animals. All experiments were conducted following standard procedures. The extract in the form of an ointment was used for evaluating the wound healing potential in excision wound model. In the incision wound model, the extract was administered orally in graded doses of 100, 200 and 400 mg/kg b.w. Saba florida leaf extract demonstrated higher percentage of wound contraction in the excision wound model compared to the standard (Povidone iodine ointment) and the control. In the incision wound model healing rate was dose dependent. In both excision and incision wound models, wound surface protein increased and wound surface microbial load decreased after days. The healing or wound contraction elicited by Saba florida leaf extract in this investigation following topical and oral administration strongly support the verbal claim of traditional doctors on the use of this plant.

Keywords: Saba florida, wound healing, skin ulcer, Ibaji, Rattus novergicus, and ointment.

INTRODUCTION

Various plant species have served as a source of medicine for people all over the world, for years plant is one of the most intense areas of natural product research yet the field is far from being exhausted. Plants and their extracts have immense potential for the management and treatment of wound. The phytomedicine for wound healing are not only cheap and affordable but are also purportedly safe as hypersensitive reactions are rarely encountered with the use of these agents. These natural agents induce healing and regeneration of the tissue by multiple mechanisms, however, there is need for scientific validation, standardization and safety evaluation of plants of traditional medicine before they could be recommended for healing of wounds [1].

Wound is defined as the disruption of the cellular and anatomic discontinuity of a tissue [1]. Wound may be produced by chemical, physical, thermal, microbial or immunological insult to the tissue. Wound cause discomfort and are more prone to infection and other troublesome complications [2]. Some diseases like diabetes, immunocompromised conditions, ischaemia and conditions like malnourishment, ageing, local infection, local tissue damage due to burn or gunshot often leads to delay in wound healing. Infection is the major complications of burn injury and is responsible for 50-75% of hospital deaths [3]. Wound healing consists of an orderly progression of events that reestablish the integrity of the damaged tissue. Many of the synthetic drugs currently used for the treatment of wounds are not only expensive but also pose problems such as allergy, drug resistance etc and this situation has forced the scientists to seek alternative drugs [4]. Efforts are being made all over the world to discover agents that can promote healing and thereby reduce the cost of hospitalization and save the patient from amputation or other severe complications.

More than 80% of the world population still depends upon traditional medicines for their ailments [5], especially for wound management [6], as they provide a moist environment to encourage the establishment of the suitable
environment. Many medicinal plants are claimed to be useful for wound healing in the traditional system of medicine though their mechanism of action and efficacy have not been evaluated scientifically.

*Saba florida* is one of the African medicinal plant which has not been fully studied scientifically. The plant is found in Ibaji and other parts of Kogi State, Nigeria. Traditionally, bark decoctions are used to treat rheumatism. The leaves are eaten as an antidote against vomiting and the bark decoctions also are administered for diarrhea and food poison [7]. Recently we have reported the antioxidant properties of *Saba florida* *invitro*. It is relatively non-toxic [7]. *Saba florida* parts were found to contain vitamins A and E, fatty acids and other nutrients [8]. Vitamin E is essential for wound healing [9].

In the light of *Saba florida’s* use in the management of diverse diseases and treatment of skin ulcer in folklore medicine, the present study was conducted to evaluate the excision and incision wound healing capacity of *Saba florida* leaf extract in experimental *Rattus novergicus*.

**MATERIALS AND METHODS**

**Collection and preparation of plant sample**

The plant sample was collected from Igboigo-umale in Ibaji Local Government Area, Eastern part of Kogi State, Nigeria during the dry season. Dirt was removed from the plant by rinsing in clean water. The leaves were air-dried for three (3) weeks and pulverized using motorized blender.

**Preparation of extract**

Cold extraction method was followed. A portion (350g) of the powdered sample was weighed into conical flask and 350 ml of pure methanol (99.9%) was added and left for 72h. The mixture was filtered under vacuum pressure and the filtrate was concentrated using rotary evaporator.

**Plant identification**

The plant was identified in the Botany unit of the Department of Biological Sciences, Kogi State University, Anyigba, Nigeria as *Saba florida* (Benth).

**Ointment preparation for topical application**

An alcohol free extract of *Saba florida* leaf gel was used for the preparation of the ointment for topical application [10]. A 10% (w/w) of extract ointment was formulated using soft white paraffin base.

**Chemicals**

All chemicals and reagents used were of analytical grade and purchased from BDH, Poole, England.

**Experimental animals**

Wister albino rats (*Rattus novergicus*), (male) of four (4) weeks, weighing between 100 and 150 g were obtained from the Department of Biochemistry, Kogi State University animal house and used for wound healing investigation. The animals were housed in standard environmental conditions of temperature (31 ±1°C), humidity (60± 0.2%) and a 12 h light and 12 h dark cycle. Rats were fed with standard rodent diet and tap water ad libitum. The study was carried out following the guidelines of the principles of laboratory animal care [12].

**Excision wound model**

The back of the animals were shaved and sterilized with 70% ethanol before 7 X 7 mm excision wound was created by a surgical blade from a predetermined shaved area on the back of each animal [13]. The wound was left undressed to the open environment and no local or systemic antimicrobial agents were used. This model was used to monitor the rate of wound contraction. The experimental groups were topically applied with the extract twice daily for consecutive 24 days. The group treated with Povidone iodine drug served as a reference. A progressive decrease in
the wound area was monitored periodically at every 8th day interval. The wound contractions were measured by a tracing paper on the wounded margin and calculated as percentage reduction in wound area. The actual value was converted into percentage value taking the size of the wound at time of wounding as 100%. The granulation tissues were removed on the 8th, 16th and 24th post wound days and analyzed for protein content (collagen). The animals were divided into 3 groups of four (4) rats each.

Group 1: served as test group treated with *Saba florida* extract ointment for 24 days.

Group 2: served as reference standard treated with wound healing ointment (Povidone iodine).

Group 3: served as negative control treated with paraffin base.

**Incision wound model**

A longitudinal para-vertebral incision of 5 cm in length was made through the entire thickness of the skin and cutaneous muscle with the help of a scalpel [14]. After complete homeostasis the wound was closed by means of interrupted sutures placed at equidistant points of 1 cm apart. The sutures were removed on the 8th post wound day and the topical application of extract ointment and oral administration of the extract continued. The animals were divided into four (4) groups of four (4) animals each.

Group 1: was treated with *S. florida* (100 mg/kg b.w).

Group 2: was treated with *S. florida* (200 mg/kg b.w).

Group 3: was treated with *S. florida* (400 mg/kg b.w).

Group 4: served as standard and treated with povidone iodine ointment topically.

**Estimation of wound surface total protein**

Total protein concentration on the regenerated tissues from the healed lesions of wound sample was determined using the method described by [15].

**Estimation of wound surface microbial load**

The wound surface microbial load was estimated based on the method described by [16].

**Statistical analyses**

All data were expressed as mean ±S.D and Graph Pad In stat –[Data Set 1.ISD] was applied to determine the significance of the difference at P<0.05.
RESULTS AND DISCUSSION

Table 1: Effects of *S. florida* leaf extract on excision wound healing in *Rattus novergicus*

<table>
<thead>
<tr>
<th>Group/treatment</th>
<th>Percentage closure of excision wound area after days</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>8</td>
</tr>
<tr>
<td>1. Treated with <em>S. florida</em></td>
<td>36.21±1.83&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2. Treated with povidone</td>
<td>21.74±4.30&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>3. Wound control</td>
<td>25.21±3.29&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
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Values are mean ±S.D (N=4). The mean value with the same superscript in the column are not significantly different (P>0.05). Values with asterisk on the same row are significantly different (P<0.05).

The topical application of *S. florida* ointment increased the percentage of wound contraction and this indicates rapid epithelizaton and collagenation. The administration of this extract (*S. florida*) accelerated the progression of wound healing by 8<sup>th</sup> day i.e. (36.21±1.83%) compared with control (25.21 ±3.29 %) (Table 1).

Table 2: Effect of *S. florida* leaf extract on excision wound protein concentration in *Rattus novergicus*

<table>
<thead>
<tr>
<th>Group/treatment</th>
<th>Percentage protein concentrations after days</th>
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<tbody>
<tr>
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</tr>
<tr>
<td>1. Treated with <em>S. florida</em></td>
<td>12.45±1.42&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>2. Treated with povidone</td>
<td>8.60±2.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3. Wound control</td>
<td>7.35±0.57&lt;sup&gt;b&lt;/sup&gt;</td>
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</table>

Values are mean ± S.D (N=4). Mean values with the same superscript are not significantly different (P>0.05)
Table 3: Effect of *S.* *florida* leaf extract on incision wound protein concentration in *Rattus novergicus*

<table>
<thead>
<tr>
<th>Group/treatment</th>
<th>Percentage protein concentrations after days</th>
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<tbody>
<tr>
<td></td>
<td>8</td>
</tr>
<tr>
<td>1. <em>S.</em> <em>florida</em> (100mg/kg b.w)</td>
<td>13.88±3.94(^b)</td>
</tr>
<tr>
<td>2. <em>S.</em> <em>florida</em> (200mg/kg b.w)</td>
<td>15.88±2.52</td>
</tr>
<tr>
<td>3. <em>S.</em> <em>florida</em> (400mg/kg b.w)</td>
<td>17.92±6.27(^a)</td>
</tr>
<tr>
<td>4. Wound control</td>
<td>7.35±0.57(^ab)</td>
</tr>
</tbody>
</table>

Values are mean ±S.D (N=4). Mean values with the same superscript are significantly different (P<0.05)

Table 4: Effect of *S.* *florida* on excision wound surface microbial load (CFU/ml)

<table>
<thead>
<tr>
<th>Group/treatment</th>
<th>Microbial count after days</th>
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<tbody>
<tr>
<td></td>
<td>8</td>
</tr>
<tr>
<td>1. <em>S.</em> <em>florida</em> extract</td>
<td>64</td>
</tr>
<tr>
<td>2. Povidone iodine</td>
<td>74</td>
</tr>
<tr>
<td>3. Wound control</td>
<td>84</td>
</tr>
</tbody>
</table>

Microbial load decreased with days of application of the extract.

Wound healing process consists of different phases such as granulation, collagenationation, collagen maturation and scar maturation which are concurrent but independent of each other. It is a complex and dynamic process of...
restoring cellular structures and tissue layers in damaged tissue as closely as possible to its normal state. Wound contracture is a process, commencing in the fibroblastic stage whereby the area of the wound undergoes shrinkage. Collagen, the major component which strengthens and supports extracellular tissue is composed of amino acids, hydroxyproline, which has been used as a biochemical marker for tissue collagen [17]. Hence in this investigation two models were used to assess the effect of the alcoholic extract of S.florida leaf. The result of the present investigation showed that S.florida possesses a definite pro-healing action.

In excision wound healing model the alcoholic extract of the leaf of the plant S.florida showed significant increase in percentage closure of excision wounds. Higher percentage of wound closure was observed in the group of animals treated with S.florida on day 24 of the experiment (Table 1) compared to the standard. In similar manner percentage protein concentration increased but much more in group 1 treated with S. florida. The observed increase in protein (Table 2), an important constituent of extracellular matrix in the treated animals confirmed that the extract had positive effects towards cellular proliferation, granulation tissue formation and epithelization. The increase in protein content in the treated group is predominantly due to enhanced collagen synthesis in the S.florida gel extract treated group. The capacity to form supramolecular aggregates in extracellular spaces is one of the important characteristics of molecules belonging to the collagen family of proteins [18]. The importance of protein in wound healing has been appreciated for a long time for the simple reason that the ultimate result of most repairs in the higher vertebrates is the formation of scar tissue composed of collagenous fibers.

As presented on table 2, the S. florida treated group had sharp increase in protein content from day 8 to 24. This is comparable to the standard povidone iodine used. The present investigation demonstrated that alcoholic extract of S.florida has properties that render it capable of promoting wound healing activity compared with standard treatment controls. In the incision

Wound model wound surface protein was dose dependent. It was observed that combination of topical and oral administration could be more effective in the treatment of wound using S.florida extract. The results are also comparable with the standard. Post operative wounds are commonly known to be complicated by infection. Earlier studies have shown that antimicrobial activity of various plants supports wound healing. As presented on tables 4 and 5, the wound surface microbial load decreased with days of treatment. This is an indication that the plant extract possessed antimicrobial activity. In the incision wound model (Table 5), the reduction of wound surface microbial load was also dose dependent; hence this present research supports traditional claims of the plant in wound healing. Thus it may be concluded that S.florida leaf gel extract have the potential to satisfy all requirements of an ideal dressing material in that it provides an environment at the surface of the wound in which healing took place at the maximum rate consistent with the formation of granulation tissue with an acceptable cosmetic appearance and also provides a rationale for the use of S.florida preparations in the traditional system of medicine to promote wound healing.

Furthermore, it can be concluded that the S.florida extract has a beneficial effect as antiseptic and as an injury –healing promoter. This effect may be explained by several mechanisms such as coating the wound, forming complexes with proteins of microorganism cell wall, chelating free radicals and reactive oxygen species, stimulating the contraction of the wound and increasing the formation of new capillaries and fibroblasts. Moreover, the extract did not produce any adverse effect and because of this it is possible to recommend its use in the treatment of skin wounds or ulcers.

ACKNOWLEDGEMENT

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REFERENCES


