Evaluation of antimicrobial and wound-healing potential of *Catharanthus roseus* leaf extract in rats as animal model

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Abstract

**Background:** *Catharanthus roseus* L. is native to the Caribbean Basin and has historically been used to treat a wide assortment of diseases. The objective of this study was to evaluate the antimicrobial and wound healing activity of leaf extract of *Catharanthus* in rats.

**Methods:** The ethanol extract of the leaves of *C. roseus* was applied topically to gender-matched Sprague Dawley rats (100 mg kg⁻¹ day⁻¹) for 10 days, and was studied for its wound healing, using excision and incision wound model. The animals were divided into three groups of 6 each in both models. Group 1 animals were treated with carboxymethyl cellulose (1% in ethanol) as placebo control and group 2 animals the reference standard control were treated with sulphathiazole ointment. Animals in group 3 were treated with the ethanol extract of *C. roseus*. Healing was assessed by the rate of wound contraction, period of epithelialisation, tensile strength (skin breaking strength), granulation tissue weight and hydroxyproline content.

**Results:** Experimental animals treated with the extract of *C. roseus* had high rate of wound contraction (p<0.001), increased skin breaking strength, decrease in the period of epithelialisation, significant increase in dry weight (p<0.001) and hydroxyproline content (p<0.001) of the granulation tissue when compared with the controls. The *Pseudomonas aeruginosa*, *Enterobacter agglomerans* and *Staphylococcus aureus* were sensitive against the leaf extract of *C. roseus*

**Conclusion:** Wound contraction together with the increased tensile strength, hydroxyproline content and the antimicrobial activity support the use of *C. roseus* in topical management of wound healing.
Background
The therapeutic efficacies of many indigenous plants, for various diseases have been described by traditional herbal medicine practitioners [1]. Natural products are a source of synthetic and traditional herbal medicine and are still the primary health care system [2]. The past decade has seen considerable change in opinion regarding ethnopharmacological therapeutic applications. The presence of various life sustaining constituents in plants has urged scientists to examine these plants with a view to determine potential wound healing properties.

Wound healing is the process of repair that follows injury to the skin and other soft tissues. Following injury, an inflammatory response occurs and the cells below the dermis (the deepest skin layer) begin to increase collagen (connective tissue) production. Later, the epithelial tissue (the outer skin layer) is regenerated. There are three stages to the process of wound healing: inflammation, proliferation, and remodeling. The proliferative phase is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelialisation and wound contraction. Angiogenesis involves new blood vessel growth from endothelial cells. In fibroplasia and granulation tissue formation, fibroblasts excret collagen and fibronectin to form a new, provisional extracellular matrix. Subsequently epithelial cells crawl across the wound bed to cover it and the wound is contracted by myofibroblasts, which grip the wound edges and undergo contraction using a mechanism similar to that in smooth muscle cells.

*Catharanthus roseus* L. (apocyanaceae) also known as *Vinca Rosea*, is native to the Caribbean Basin and has historically been used to treat a wide assortment of diseases. European herbalists used the plant for conditions as varied as headache to a folk remedy for diabetes. It has more than 400 known alkaloids, some of which are approved as antineoplastic agents to treat leukemia, Hodgkin's disease, malignant lymphomas, neuroblastoma, rhabdomyosarcoma, Wilms' tumor, and other cancers. Its vasodilating and memory-enhancing properties have been shown to alleviate vascular dementia and Alzheimer’s disease [3, 4]. The two classes of active compounds in *Vinca* are alkaloids and tannins. The major alkaloid is vincamine and its closely related semi-synthetic
derivative widely used as a medicinal agent, known as ethyl-apovincaminate or vinpocetine, has vasodilating, blood thinning, hypoglycemic and memory-enhancing actions [5, 6]. The extracts of Vinca have demonstrated significant anticancer activity against numerous cell types [7].

Extracts from the dried or wet leaves of plants are applied as a paste on wounds in some rural communities. The fresh juice from the leaves of C. roseus made into a tea has been used by Ayurvedic physicians in India for external use to treat skin problems, dermatitis, eczema and acne. There is no previous report on wound healing activities of C. roseus in literature to the best of our knowledge and in this paper, we report for the first time, the efficacy of C. roseus leaf extract in the treatment of wounds.

Methods

Plant Material and extract preparation

The leaves of C. roseus were collected locally in April 2006 and identified by the plant taxonomist and curator, National Herbarium of Trinidad and Tobago, The University of the West Indies, St. Augustine, Trinidad and a voucher specimen was also deposited at the herbarium (specimen number: 36458). The fresh leaves were shade dried and ground into a powder using an electric blender. The fine powder (120 g) was suspended in 200 ml of ethanol for 20 hours at room temperature. The mixture was filtered using a fine muslin cloth followed by filter paper (Whatman No: 1). The filtrate was placed in an oven to dry at 40°C and clear residue was used for the study. The extract was subjected to preliminary phytochemical tests.

Animals

The study was approved by the Ethics Committee for animal experimentation (AHC06/07/1), The Faculty of Medical Sciences, The University of the West Indies, St. Augustine

Healthy inbred gender-matched Sprague Dawley rats weighing 200-220g were used for the study. They were individually housed and maintained on normal food and water ad
Animals were periodically weighed before and after experiments. The rats were anaesthetized prior to and during infliction of the experimental wounds. The surgical interventions were carried out under sterile conditions using ketamine anaesthesia (10mg/kg). Animals were closely observed for any infection and those which showed signs of infection were separated and excluded from the study and replaced.

An acute toxicity study was conducted for the extract by the stair-case method [8]. The LD$_{50}$ of ethanol leaf extract was found to be 1000 mg/kg, b.w. One tenth of the dose was selected for the evaluation of wound-healing activity i.e., 100 mg/kg, b.w.

**Wound-healing activity**

Excision and incision wound models were used to evaluate the wound-healing activity of *C. roseus*.

**Excision wound model**

Animals were anaesthetized prior to and during creation of the wounds. The rats were inflicted with excision wounds as described by Morton and Malon [9]. The dorsal fur of the animals was shaved with an electric clipper and the anticipated area of the wound to be created was outlined on the back of the animals with methylene blue using a circular stainless steel stencil. A full thickness of the excision wound of 2.5 cm (circular area = 4.9 cm$^2$) in length and 0.2 cm depth was created along the markings using toothed forceps, a surgical blade and pointed scissors. The entire wound was left open [10]. The animals were divided into three groups of 6 each. In Group 1 animals were treated with 1% CM cellulose in ethanol as a placebo control. Group 2 animals served as the reference standard control and were treated with sulphathiazole ointment. The animals of Group 3 were treated with the ethanol extract of *C. roseus* (100 mg kg$^{-1}$ day$^{-1}$) for 10 days. The parameters of efficacy studied were wound closure, time to epithelialisation, hydroxyproline content and weight of the granulation tissue. The wounds were measured on days 1, 5 and 15 post-wounding using transparency paper and a permanent marker. The wound areas recorded were measured using a graph paper. The period of
epithelialisation was calculated as the number of days required for falling of the dead tissue remnants without any residual raw wound.

The granulation tissue formed on the wounds of all the groups of animals was excised on the 11\textsuperscript{th} postoperative day and the weights recorded. The tissue was dried in an oven at 60\textdegree C and the dry weight was documented. Acid hydrolysate of the dry tissue was used for the determination of hydroxyproline [11].

\textit{Incision wound model}

As with the above model rats were anaesthetised prior to and during creation of the wounds. The dorsal fur of the animals was shaved with an electric clipper. A longitudinal paravertebral incision, six centimeters in length was made through the skin and cutaneous muscle on the back as described by Ehrlich and Hunt et al. [12]. After the incision, surgical sutures were applied to the parted skin at intervals of one centimetre. The wounds were left undressed. The extract was topically applied to the wound. The sutures were removed on the 8\textsuperscript{th} post wound day and the application of extract was continued. The skin-breaking strength was measured on the 10\textsuperscript{th} day by the method described by Lee [13]. The healing tissue was taken on the day 11 for histological studies.

\textbf{Antimicrobial activity}

Known organisms (\textit{Pseudomonas aeruginosa}, \textit{Beta-hemolytic streptococci}, \textit{Enterobacter agglomerans} and \textit{Staphylococcus aureus}) were cultured on to Mac Conkey and blood agar plates. The sensitivity testing was done using Muller Hinton Agar plates. The organisms were put up against \textit{C. roseus} extract and the plate was incubated overnight at 35-37\textdegree C. The sensitivity of the organism against the extracts was then read and noted.

\textbf{Statistical analysis}

Results, expressed as mean ± SD were evaluated using Student’s t-test and significance was set at p <0.05.
Results
In both the models studied, significant wound-healing activity was observed in animals treated with the *C. roseus* leaf extract compared with those who received the reference standard and placebo control treatments. Table 1 shows the effects of the ethanolic extract *C. roseus* leaf applied topically at a dose of 100 mg kg\(^{-1}\) day\(^{-1}\) for 10 days on wound healing activity in rats. In the excision wound model, *C. roseus* treated animals showed a significant reduction in the wound area (p< 0.001), faster rate of epithelialisation (10.2 ± 0.13) and significantly increased hydroxyproline content (p< 0.05) as compared with the two control groups of animals.

In the incision wound model, *C. roseus*-treated animals demonstrated high skin-breaking strength up to 440.0 ± 4.53. A significant increase was observed in the weight (p< 0.01) and hydroxyproline content (p< 0.05) of the granulation tissue [Table 2] in the group 3 animals treated with the extract.

The phytochemical analysis of the *C. roseus* by thin layer chromatography showed the presence of polyphenols, triterpenoids, and alkaloids. The microbial organisms including *Pseudomonas aeruginosa*, *Enterobacter agglomerans* and *Staphylococcus aureus* were sensitive against *C. roseus*. However, the Beta hemolytic streptococci were resistant against extract.

Discussion
Wound healing 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 that occurs throughout the healing process, commencing in the fibroblastic stage whereby the area of the wound undergoes shrinkage. It has 3 phases; inflammatory, proliferative and maturational and is dependent upon the type and extent of damage, the general state of the host’s health and the ability of the tissue to repair. The inflammatory phase is characterized by hemostasis and inflammation, followed by epithelialization, angiogenesis, granulation tissue formation, and collagen deposition in
the proliferative phase. In the maturational phase, the final phase of wound healing the wound undergoes contraction resulting in a smaller amount of apparent scar tissue.

Granulation tissue formed in the final part of the proliferative phase is primarily composed of fibroblasts, collagen, edema, and new small blood vessels. The increase in dry granulation tissue weight in the test treated animals suggests higher protein content. The ethanol extract of *C. roseus* demonstrated a significant increase in the hydroxyproline content of the granulation tissue indicating increased collagen turnover. Collagen, the major component which strengthens and supports extra cellular tissue is composed of the amino acid, hydroxyproline, which has been used as a biochemical marker for tissue collagen [14].

The preliminary phytochemical analysis of the leaf extract showed the presence of polyphenols, triterpenoids and alkaloids. Any one of the observed phytochemical constituents present in *C. roseus* may be responsible for the wound healing activity. Recent studies have shown that phytochemical constituents like flavanoids [15] and triterpenoids [16] are known to promote the wound-healing process mainly due to their astringent and antimicrobial properties, which appear to be responsible for wound contraction and increased rate of epithelialisation. Our earlier studies showed the presence of triterpenoids which were responsible for the effective wound healing activity of *Cecropia peltata* [17] and *Pentas lanceolata* [18].

The wound-healing property of *C. roseus* may be attributed to the phytoconstituents present in the plant, and the quicker process of wound healing could be a function of either the individual or the additive effects of the phytoconstituents. The early tissue approximation and increased tensile strength of the incision wound observed in our study may have been contributed by the tannin phytoconstituent of *C. roseus* from the astringent effect which has been reported elsewhere [19] Further phytochemical studies are in progress to isolate, characterize and identify the specific active compounds in this plant responsible for wound healing activity. Electron microscopic examination will yield the effect of the extract on angiogenesis, epithelialisation or collagen deposition. We plan
to conduct additional studies to examine the constituent phytochemical constituents which contribute to the pharmacological activity of *C. roseus*. Wound healing activity of plant extracts may also be subsequent to an associated anti-microbial effect [20]. Our further studies will explore the anti-microbial effect of the extract and the specific phase of wound healing using electron microscopy.

**Conclusion**

The present study has demonstrated that an ethanol extract of *C. roseus* has properties that render it capable of promoting accelerated wound healing activity compared with placebo and standard treatment controls. Wound contraction, increased tensile strength, increased hydroxyproline content and antimicrobial activity support further evaluation of *C. roseus* in the topical treatment and management of wounds.

**Competing interests**

The author(s) declare that they have no competing interests

**Acknowledgements**

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**Authors’ contributions**

SN was responsible for designing and the entire work
AM contributed for phytochemical study
LMP was responsible for pharmacological aspects of the experiments

**References:**


17. Shivananda Nayak B: **Cecropia peltata L (Cecropiaceae) Has Wound Healing potential-A preclinical study in Sprague Dawley Rat model.** *International Journal of Lower Extremity Wounds*. 2006, **5**: 20-26


Table 1: Wound healing effects of ethanolic extract of *C. roseus* leaves rats (excision wound)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo control</th>
<th>Standard control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wound area (mm²):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>220.3 ± 23.80</td>
<td>220.0 ± 23.83</td>
<td>222.50 ± 14.7</td>
</tr>
<tr>
<td>Day 5</td>
<td>173.6 ± 22.8</td>
<td>169.0 ± 6.14</td>
<td>163.16 ± 31.58</td>
</tr>
<tr>
<td>Day 15</td>
<td>130.8 ± 26.90</td>
<td>63.5 ± 25.40</td>
<td>66.50 ± 22.0 **</td>
</tr>
<tr>
<td>Period of epithelialisation</td>
<td>14.1 ± 0.12</td>
<td>9.8 ± 0.14</td>
<td>10.20 ± 0.13 **</td>
</tr>
<tr>
<td>Hydroxyproline (mg g⁻¹)</td>
<td>24.1 ± 6.11</td>
<td>57.1 ± 1.73</td>
<td>64.00 ± 29.85 **</td>
</tr>
</tbody>
</table>

100 mg kg⁻¹ day⁻¹ p.o

*N* = 6

Values are expressed as mean ± SD

*p* < 0.05 and **p** < 0.001 vs. control. Independent *t*-test
Table 2: Wound healing effects of ethanolic extract of *C. roseus* leaves rats (incision wound)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo control</th>
<th>Standard control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin breaking strength (g)</td>
<td>319.13 ± 3.23</td>
<td>470.5 ± 4.10</td>
<td>435.0 ± 4.53**</td>
</tr>
<tr>
<td>Wet weight granulation</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>tissue of the (mg)</td>
<td>79.3 ±19.20</td>
<td>80.2 ±14.20</td>
<td>88.60 ±18.20</td>
</tr>
<tr>
<td>Dry weight of the granulation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tissue (mg)</td>
<td>7.5 ± 1.22</td>
<td>15.0 ± 0.15</td>
<td>12.60 ± 1.90**</td>
</tr>
<tr>
<td>Hydroxyproline (mg g⁻¹)</td>
<td>26.5 ± 6.83</td>
<td>120.1 ± 25.32</td>
<td>110.30 ±19.47**</td>
</tr>
</tbody>
</table>

100 mg kg⁻¹ day⁻¹ p.o

*N = 6*

Values are expressed as mean ± SD

*p < 0.05 and **p<0.001 vs. control. Independent *t*- test