

## Anti-microbial and wound healing activities of *Cordia macleodii* Hook. f & Thoms. leaves

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The present study was carried out to evaluate anti-microbial and wound healing activities of leaves of *Cordia macleodii* Hook. f & Thoms, a folklore medicinal plant. Anti-microbial activity was evaluated against clinically important bacteria, yeast and fungal strains using agar disc diffusion method. Excision and incision wound models were used to evaluate the wound healing activity on Wistar strain albino rats. The effects of test drug on the rate of wound healing were assessed by the rate of wound closure, period of epithelialisation, tensile strength and histopathology of the granulation tissue as well as incisional skin area. The study shows test drug is moderately sensitive against Gram positive bacteria and has no action against Gram negative bacteria. Analysis of the results generated in wound healing activity which involved studying the impact of local application of *C. macleodii* on excision wound, incision wound and dead space wound, show that it has no influence over excision wound contraction. It has weak tensile strength promoting property in incision wounds and neo-vascularization and ground substance formation in the dead space wounds.

**Keywords:** Anti-microbial, *Cordia macleodii*, Ethnomedicine, Wound healing.

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### Introduction

A wound is a disruption of tissue integrity that results in damage and is typically associated with loss of function. Wound healing can be defined as a complex dynamic process that results in the restoration of anatomic continuity and function. It is a finely orchestrated and overlapping sequence of events involving: control of infection, resolution of functional connective matrix, contraction, resurfacing, differentiation and remodeling<sup>1</sup>.

Medicinal plants have great potentials and have been shown to be very beneficial in wound care, promoting the rate of wound healing with minimal pain, discomfort and scarring to the patient<sup>2</sup>. Some of these plants owe their effects to direct effect on the wound healing processes and some to their anti-microbial properties. A combination of these properties is also possible in some of the medicinal plants used in wound healing.

*Cordia macleodii* Hook. f & Thoms. belonging to the family Boraginaceae is reported for its wonderful wound healing, aphrodisiac and mouth sores healing

activities<sup>3,4</sup>. Macerated fresh leaves of the plant have been used by the people residing in and around the forest of Bargarh district, Orissa, India for checking bleeding from cut injuries and rapidly healing the same. The tribals have been using it since time immemorial. It is said that they used the leaves for packaging the meat to carry it home and found that the blood had coagulated in the minced meat. Since then they have been using it for healing cuts and wounds in paste form of fresh leaves or some times in the form of powder<sup>4</sup>. However, there is not enough scientifically proven data to support the wound healing and anti-microbial activities of *C. macleodii* and also on other biological activities on this plant in literature except hepatoprotective activity<sup>5</sup>. The present study was undertaken to explore the antimicrobial and wound healing effects of *C. macleodii* leaf powder.

### Materials and Methods

#### Plant material

The fresh leaves of the plant were collected from its natural habitat of Bolangir, Orissa, India (Plate 1), in the month of November 2008. The plant was

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Plate 1 — *Cordia macleodii*: a- plant, b- twig with leaves, c- leaves

carefully identified and authenticated by taxonomist, with the help of flora of Orissa, prior to the collection of samples. A voucher specimen has been preserved in the herbarium attached to our Institute. The collected leaves were washed, shade dried and pulverized and sieved through 80 mesh and preserved in an airtight vessel for experimental studies.

#### Test microorganisms and growth media

Gram positive and Gram negative bacteria, yeasts and molds were used for anti-microbial activity studies. Gram positive bacteria include *Streptococcus* and *Staphylococcus aureus*, whereas Gram negative bacteria include *Klebsiella pneumoniae* and *Escherichia coli*. Yeast included *Candida albicans*, whereas molds included *Aspergillus niger*.

These organisms were identified and procured from Shrey Pathology Laboratory (ISO 9001:2008 certified), Jamnagar, India. The bacterial strains were grown in Mueller–Hinton agar (MHA) plates at 37°C, whereas the yeasts and molds were grown in Sabouraud dextrose agar (SDA) and potato dextrose agar (PDA) media, respectively, at 28°C. The stock cultures were maintained at 4°C.

#### Anti-microbial assay

Antibacterial and antifungal activities of plant leaf powder were investigated by the disk diffusion method<sup>6</sup>; 0.6 ml of standardized bacterial stock suspensions ( $10^8$  colony forming units per ml) was thoroughly mixed with 60 ml of sterile nutrient agar. 20 ml of the inoculated nutrient agar were distributed into sterile petri dishes. The agar was left to set and in each of these plates 4 cups, 10 mm in diameter, was cut using a sterile cork borer No.4 and the agar discs were removed. Cups were filled with 100 mg of test drug and allowed to diffuse at room temperature for two hours. The plates were incubated in the upright position at 37°C for 18 hours. After incubation the diameter of the results and growth inhibition zones were measured, averaged and the mean values were recorded.

#### Wound healing activity tests

##### Animals

Wistar strain albino rats of either sex weighing between 180-200g were selected from the animal house attached to our institute. They were housed at  $22 \pm 03^\circ\text{C}$  with constant humidity of 50-70% on a

12 h natural day and night cycles. They were fed with diet Amrut brand rat pellet food supplied by Pranav Agro Industries, Baroda and tap water was given *ad libitum*. The animals were left for 3 days at room conditions for acclimatization. The study was permitted by the Institutional Animal Ethics Committee (IAEC 09-10/05/MD/01).

#### **Excision wound model**

Prior to the operative procedure all the instruments (scissor, forceps, etc.) were autoclaved. The area to be excised (on the back portion of the rat – suprascapular region) was shaved carefully by scissor prior to the procedure without causing any abrasions. The rats were anaesthetized with diethyl ether and they were inflicted with excision wounds as described by Morton and Malone (1972)<sup>7</sup>. The dorsal fur of the animals was shaved and the area of the wound to be created was outlined on the back of the animals with marker using a circular coin. A full thickness of the excision wound of circular area  $380 \pm 40\text{mm}^2$  and 2 mm depth was created along the markings with a surgical blade. The animals were randomly divided into three groups of six each. First group (Group A) is served as normal control to which normal saline was applied. To the second group (Group B) test drug in the form of fine powder (about 100 mg) was applied. Third group (Group C) was applied with standard drug (Betadine) daily until complete epithelialization. The wound contraction rate was assessed by tracing the wound on every third day using transparency paper and a permanent marker. The wound areas recorded were measured using a graph paper. The point at which the eschar fell off without any residual raw wound was considered epithelialization.

#### **Incision and dead space wound model**

The effect of test drug on incision and dead space wound was evaluated by noting effect on the formation of granulation tissue in subcutaneously implanted PVC tube. The selected animals were randomly divided into three groups of six each as mentioned in excision wound model. The animals were anaesthetized by inhalation of diethyl ether and a mid line incision of 3 cm was made and a tunnel was created subcutaneously in which sterilized PVC tube (2 cm length and 1 mm diameter) was inserted and the incision was closed with the help of two interrupted sutures at 1 cm apart as described by Ehrlich and Hunt (1968)<sup>8</sup>. The test drug was filled

in the PVC tube before implantation and also applied locally over the incision wound daily for 12 consecutive days. The sutures were removed on the 8<sup>th</sup> post wound day and the skin-breaking strength was measured on the 12<sup>th</sup> day by the method described by Lee (1968)<sup>9</sup>. The anesthetized animal was secured to the table and a line was drawn on either side of the wound 3 mm away from the suture line. The line on either side of the suture was gripped with a forceps one at each end opposed to each other. One end of the forceps was supported firmly, whereas the other was connected to a freely suspended lightweight measuring jar. Water was slowly added continuously till the wound began to gap. As soon as wound gaping appeared the addition of water was stopped. The volume of water was determined and noted as a measure of breaking strength in grams. The PVC tube was taken out from the subcutaneous tunnel by careful dissection and the tissue was collected from the plastic tube and preserved for histopathological study. Along this the tissue of incision wound was also preserved for histopathological study. The histopathological slides of skin and granulation organs were prepared by referring standard procedures and three types of staining was carried out, viz. H and E staining<sup>10</sup>, Prussian blue and Van-Gieson's<sup>11</sup>. The slides were viewed under binocular research Carl-Zeiss's microscope (Germany) at various magnifications to note down the changes in the microscopic features of the tissues studied.

#### **Statistical analysis**

Results of wound healing activity were presented as Mean  $\pm$  SEM, difference between the groups was statistically determined by paired and unpaired student's 't' test for paired and unpaired data, respectively with the level of significance set at  $P < 0.05$ . The level of significance was noted and interpreted accordingly.

## **Results and Discussion**

#### **Anti-microbial activity**

The results reveal that *C. macleodii* has a zone of inhibition at 3 mm for both *Streptococcus* and *Staphylococcus aureus*, which is moderately sensitive while the test drug is resistant to Gram negative bacteria, viz. *Klebsiella pneumoniae* and *Escherichia coli*. Further, test drug is poorly sensitive to yeast and molds with the inhibition zone of 1 mm each.

Table 1 — Actual wound contraction (wound area in sq mm) recorded during the study on excision wound

Groups	0 day	3 <sup>rd</sup> day	6 <sup>th</sup> day	9 <sup>th</sup> day	12 <sup>th</sup> day	15 <sup>th</sup> day	% change 0-15	Days taken for complete epithelialization
Group A	425.60 ± 40.54	327.34 ± 28.02	271.22 ± 24.33	135.54 ± 17.52	65.67 ± 18.89	34.27 ± 9.54	90.86 ± 2.036	28.50 ± 02.21
Group B	406.63 ± 34.76	335.72 ± 10.94	242.43 ± 18.33	133.32 ± 19.79	73.97 ± 13.13	45.66 ± 07.7	88.78 ± 02.37	29.17 ± 02.02
Group C	428.09 ± 15.22	301.31 ± 25.93	261.79 ± 23.66	163.01 ± 35.2	86.09 ± 25.34	36.24 ± 9.90	91.23 ± 2.651	27.50 ± 02.17

The data is expressed as Mean ± SEM.

#### Effect on excision wound

In the early days (up to 3 days) moderately higher rate of contraction was observed with betadine treated group whereas it was observed slightly less in test drug applied group. So, not much difference was noted among different groups. Around 28 days were required for complete healing in control group and in betadine treated group 27 days and around 29 days were required in test drug applied group (Table 1).

#### Effect on incision wound and dead space wound

Application of test drug non-significantly increased the tensile strength of the incision wound in comparison to control group. Table 2 shows effect on test drug on tensile strength of the incision wound.

The injured skin remains vulnerable to invasive microbial infections of all kinds and subsequent development of wound sepsis until complete epithelial repairs has occurred. Bacteria directly invade wounds producing inflammation and fluid exudation which interferes with the healing process. The bacteria toxins cause tissue damage and delay fibroplasia as well as collagen synthesis<sup>12</sup>. The goal of topical anti-microbial therapy in wound care is to control microbial colonization and subsequent proliferation thus promoting the healing of the wounds<sup>13</sup>.

The bacteria that usually infect the wound are *Staphylococcus*. The study reveals moderate sensitivity of the test drug against *Staphylococcus aureus* and *Streptococcus* bacteria. This may be the one of the contributing factor for healing of wounds when applied.

Healing of skin wounds is a complex process which recruits the collaborative efforts of different tissues of varying cell lineage. The behaviour of each of the contributing cell types during the phases of proliferation, migration, matrix synthesis and contraction, as well as the growth factor and matrix signals present at a wound site are now well understood<sup>14</sup>. Following an injury, a series of

Table 2 — Effect on tensile strength of incision wound

Groups	Tensile strength (g/100g body weight)
Group A	279.88 ± 54.76
Group B	318.82 ± 42.04
Group C	278.69 ± 63.66

The data is expressed as Mean ± SEM.

events takes place in a predictable fashion to repair the damage. In the subsequent inflammatory response following an injury the cells below the dermis (the deepest skin layer) begin to increase collagen (connective tissue) production reaching the last stage of regeneration of epithelial tissue (the outer skin layer)<sup>15</sup>.

The most important component in this model is the wound closure. The efficacy of the medication is measured in terms of rate of wound contraction and duration required for complete epithelialization of the wound. The results obtained indicate that the local application of *C. macleodii* may not have much influence on the healing of the excision wound. It would be interesting to note the effect of its oral administration though not resorted to by the folklore practitioners.

In incision wound model effect of local application on two important parameters was assessed, i.e. effect of epithelialization of the incisional wound and tensile strength of the wound. The first part of evaluation was through histological evaluation of the incision wound area with different staining methods. The second part was through actual measurement of the tensile strength of wound through well established and standardized method in the laboratory. General pattern assessment is possible through standard Hematoxylin and Eosin staining; Von-Gieson stain is helpful in assessing the collagen content and Prussian blue staining is helpful in assessing the development of ground substance.

The microscopic examination of incisional wound sites showed that Betadine promotes epithelial formation. The effect of *C. macleodii* was not that

significant; increase was observed in some rats and no effect on others. Van-Gieson staining did not show any remarkable increase in the collagen content of the skin in *C. macleodii* applied groups. In Betadine group slightly higher collagen content was observed. Prussian blue staining showed less intense staining in both test drug and reference standard

applied groups indicating they do not promote formation of ground substance to significant extent. From the above it can be suggested that the local application of the test drug has only weak effect on epithelial formation in the incision wounds and may not have significant impact on the collagen and ground substance formation.

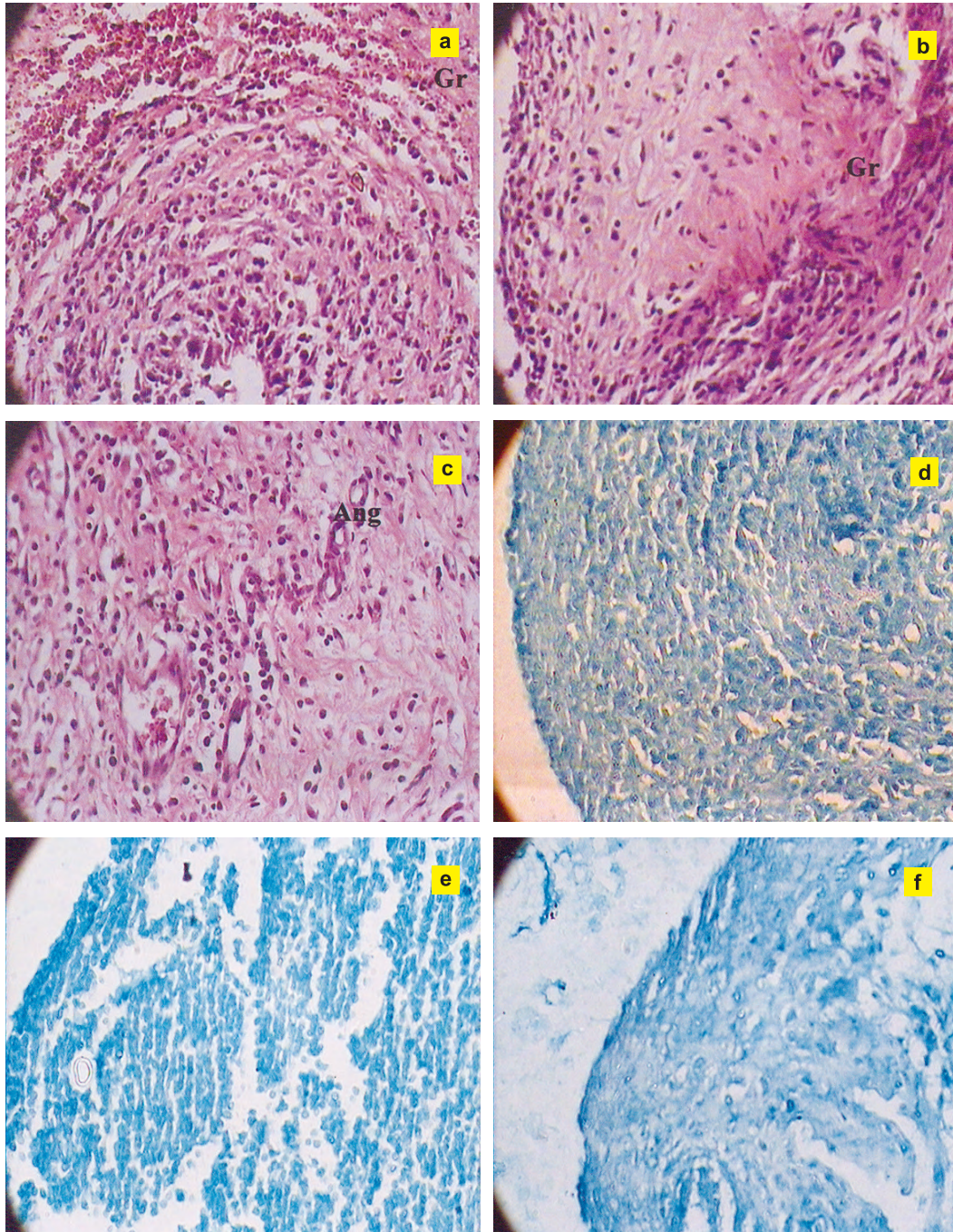


Plate 2 (a-f) — Photomicrographs of granulation tissue (1x400 magnification): a- Control group (HE stain), Gr-Granulation; b- *Cordia macleodii* treated (HE stain); c- Betadine treated (HE stain), Ang-Angiogenesis; d-Control group (P blue stain); e- *Cordia macleodii* treated (P blue stain); f- Betadine treated (P blue stain)

Effect of test drug on dead space wound was evaluated by noting down the effect of drug application (local as well as embedding in the tube) on the formation of granulation tissue in the implanted PVC tube. Surprisingly, contrary to the expectation not much granulation tissue formation could be observed in both *C. macleodii* and Betadine applied groups. Hence, the emphasis was laid on histological examination of the granulation tissue (Plate 2 a-f). H and E staining showed higher degree of angiogenesis in Betadine applied group (Plate 2c). In *C. macleodii* treated group much higher angiogenesis, cellularity and fibre content was observed in comparison to control group sections (Plate 2b). This indicates that the test drug may have some effect in the treatment of dead space wound through promotion of angiogenesis. Von-Gieson staining showed higher collagen content in Betadine treated group and in *C. macleodii* applied group the collagen content was similar to control group. The intensity of Prussian stain was found to be higher in both *C. macleodii* (Plate 2e) and betadine (Plate 2f) applied groups. From the above observed profile it can be suggested that the test drug may have angiogenesis and ground substance promotion effect which could be useful in the treatment of dead space wounds.

### Conclusion

Analysis of the results generated in the present study, which involved studying the impact of local application of *C. macleodii* on excision wound, incision wound and dead space wound, show that it has no influence over excision wound contraction. It has weak tensile strength promoting property in incision wounds and neo-vascularization and ground substance formation in the dead space wounds. The observed effect may not be sufficient to obtain the desired result and it may be necessary to provide oral drug administration as well as other pharmaceutical formulations for obtaining good results.

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