

## WOUND HEALING ACTIVITY OF AQUEOUS EXTRACTS OF LEAVES AND ROOTS OF *COLEUS AROMATICUS* IN RATS

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**Abstract:** Present study was aimed to evaluate the wound healing activity of aqueous extract of leaves and roots of *Coleus aromaticus* using excisional wound model in albino rats. The aqueous extracts were prepared using maceration method and were applied as 5% and 10% ointment. The wound healing activity of these extracts was compared with a standard drug povidone-iodine ointment. The healing tissue was also tested for tensile strength, hydroxyproline content and protein content. The histopathological examination of healing tissue was also performed. Ten percent ointment of aqueous extract of root showed complete epithelization after 12 days ( $p < 0.01$ ) and 5% ointment of leaf extract showed complete healing after 16 days ( $p < 0.01$ ).

**Keywords:** wound healing, tensile strength, hydroxyproline content, *Coleus aromaticus*

*Coleus aromaticus* Benth. (Lamiaceae) syn. *Coleus ambonicus* (Lour.) Spreng or *Plectraanthus ambonicus* is commonly known as Indian country borage. It is a large succulent herb with aromatic leaves. The leaves of this plant are traditionally used for the treatment of severe bronchitis, asthma, diarrhoea, epilepsy, vaginal discharge, renal and vesical calculi and fever. It is reported to act as an antilithotic, chemopreventive, antiepileptic and antioxidant (1, 2). The juice of leaves has been used in wound healing and swelling disorder by the tribal people. Major chemical constituents of *Coleus aromaticus* leaves include essential constituents like carvacol, thymol, eugenol, chavicol, ethyl salicylate etc. It also contains chlorophyllin, flavonoids like cirsimaritin, sitosterol-D-glucoside and also high amount of ascorbic acid (2). The plant has many uses but the wound healing potential of this plant is yet to be explored. Thus, *Coleus aromaticus* has been selected for this study.

### MATERIALS AND METHODS

#### Collection and extraction (3, 4)

The plant *Coleus aromaticus* Benth. (Lamiaceae) consisting of leaves and root was obtained from botanical garden of Barkatullah University, Bhopal (M.P.), India and was authenti-

cated at the Department of Pharmacognosy, I P S College of Pharmacy Gwalior (M.P.), India. The prepared herbarium was deposited in the Phytochemistry lab of G. R. Medical College Gwalior (M.P.), India for further reference. The leaves and roots, were separated and washed properly. These were cut into pieces before being subjected separately to cold maceration for seven days. The solvent used was distilled water. After seven days, the macerates were filtered with muslin cloth and concentrated using rotary evaporator. The extracts were tested positive for the presence of flavonoids, carbohydrates, sterols and glycosides using standard qualitative tests (5).

#### Preparations of ointment

Five and 10% ointments of aqueous leaf and root extracts were prepared using emulsifying ointment as base by mechanical incorporation method (6).

#### Animals

Adult albino Wistar rats of either sex weighing between 150–200 g, housed under standard conditions of temperature ( $22 \pm 2^\circ\text{C}$ ), relative humidity ( $55 \pm 5\%$ ), and light (12 h light/dark cycles) with adequate supply of water *ad libitum* and pallet diet (Pranav Agro Industries, New Delhi, India) were

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used for the study. The experimental protocol was approved by the Institutional Animal Ethics Committee (registration no. 1039/AC/07/CPCSEA) and was executed accordingly to the guidelines of Committee for the purpose of control and supervision of experiments on animals (CPCSEA).

#### Experimental protocol (7–9)

The wound healing activity was evaluated using excisional wound model. The experimental animals were divided into six groups of six animals in each group and received the following treatments. The first group served as negative control on which simple ointment was applied over the wounds. The second group (standard drug) served as positive control and povidone-iodine ointment was applied over the wounds of animals. The third, fourth, fifth, and sixth groups, were treated as test groups and 5% leaves, 10% leaves, 5% root and 10% root extract ointment, respectively, was applied over the wounds.

#### Excisional wound model

On the zero day, animals were anesthetized with anesthetic ether and placed on operation table in its natural position. An impression was made on the dorsal thoracic central region 5 mm away from the ears by using a round seal of 2.5 cm diameter as described by Morton and Malone (10). The skin of the impressed area was excised to the full thickness to obtain a wound area of about 500 mm<sup>2</sup> with the help of pointed forceps and and iris scissor. Hemostasis was achieved by packing the wound

with gelatin foam soaked in normal saline solution. The animals were then placed back into their individual cages.

The animals were treated with different strength of ointments of aqueous extract till complete epithelization occurs and on 11th day post wounding day the epithelial tissue was taken from the complete epithelized wounds of rats and their tensile strength was measured (11). The tissues were also subjected for histopathological examination. The remaining epithelized tissues were dried in desiccator under vacuum. The dried tissues were weighed and hydroxyproline and total protein content were estimated (12, 13).

#### Evaluation parameters

a. Wound area measurement; b. Percentages wound contraction; c. Period for epithelization; d. Tensile strength measurement; e. Hydroxyproline content; f. Protein content; g. Histopathological studies.

#### Wound area and wound contractions (%) measurement

The wound area was measured on 4th, 8th, 12th, 16th and 20th day and compared with the wound area on day zero. The degree of wound healing was calculated as percentage closure in wound area from original wound area using the formula:

$$\% \text{ closure} = \frac{\text{Wound area on zero day} - \text{wound area on the corresponding day}}{\text{Wound area on zero day}} \times 100$$

Table 1. Wound area measurement (mm<sup>2</sup>) and percentage wound contraction in excisional wound healing model.

Drug treatment	0 day	4th day	8th day	12th day	16th day	20th day
Control	508.61 ± 3.3	453.46 ± 6.6 (10.844)	245.60 ± 8.2 (51.711)	154.85 ± 10.0 (69.553)	65.214 ± 1.5 (87.178)	11.262 ± 1.0 (97.789)
Standard	515.82 ± 4.7	322.71 ± 6.7** (37.438)	99.81 ± 6.1** (82.199)	0.813 ± 0.11** (99.842)		
5% L	516.31 ± 4.6	399.41 ± 5.4** (22.645)	209.03 ± 4.2** (59.516)	60.42 ± 3.3** (88.298)	7.81 ± 1.7** (98.4870)	
10% L	523.21 ± 3.3	389.80 ± 3.5** (25.498)	162.28 ± 3.5** (68.983)	41.41 ± 1.7** (92.085)	2.99 ± 0.13** (99.427)	
5% R	503.55 ± 5.0	401.21 ± 5.0** (20.324)	159.33 ± 2.0** (68.358)	42.05 ± 2.4** (91.649)	5.77 ± 0.64** (98.854)	
10% R	513.02 ± 1.6	355.48 ± 3.8** (30.708)	113.69 ± 2.1** (77.839)	7.197 ± 0.29** (98.59)		

Values are the mean ± SE, (n = 6), ANOVA followed by Dunnett's multiple comparison test. p-values : p < 0.01\*\* as compared with control. 5% L = 5% leaves extract ointment. 10% L = 10% leave extract ointment, 5% R = 5% root extract ointment. 10% R = 10% root extract ointment.

Table 2. Tensile strength, OH-proline content, protein content, period of epithelization in excisional wound healing model.

Drug Treatment	Tensile strength (g)	OH-proline content (mg/g)	Protein content (mg/g)	Period of epithelization (days)
Control	347.750 ± 11.0	22.996 ± 1.8	30.441 ± 1.8	22.5 ± 0.56
Standard	617.166 ± 13.0**	71.513 ± 5.0**	135.783 ± 5.0**	12.5 ± 0.34**
5% L	445.916 ± 11.0**	31.375 ± 2.5**	47.749 ± 2.5**	18.166 ± 0.31**
10% L	534.416 ± 9.7**	45.491 ± 3.3**	57.934 ± 3.3**	15.666 ± 0.33**
5% R	523.583 ± 13.0**	43.032 ± 2.5**	61.709 ± 2.5**	15.833 ± 0.31**
10% R	603.583 ± 4.8**	54.098 ± 2.4**	87.065 ± 2.4**	13.666 ± 0.33**

Values are the mean ± SE (n =6), ANOVA followed by Dunnett's multiple comparison test. p-values \*\*: p < 0.01 as compared with control.

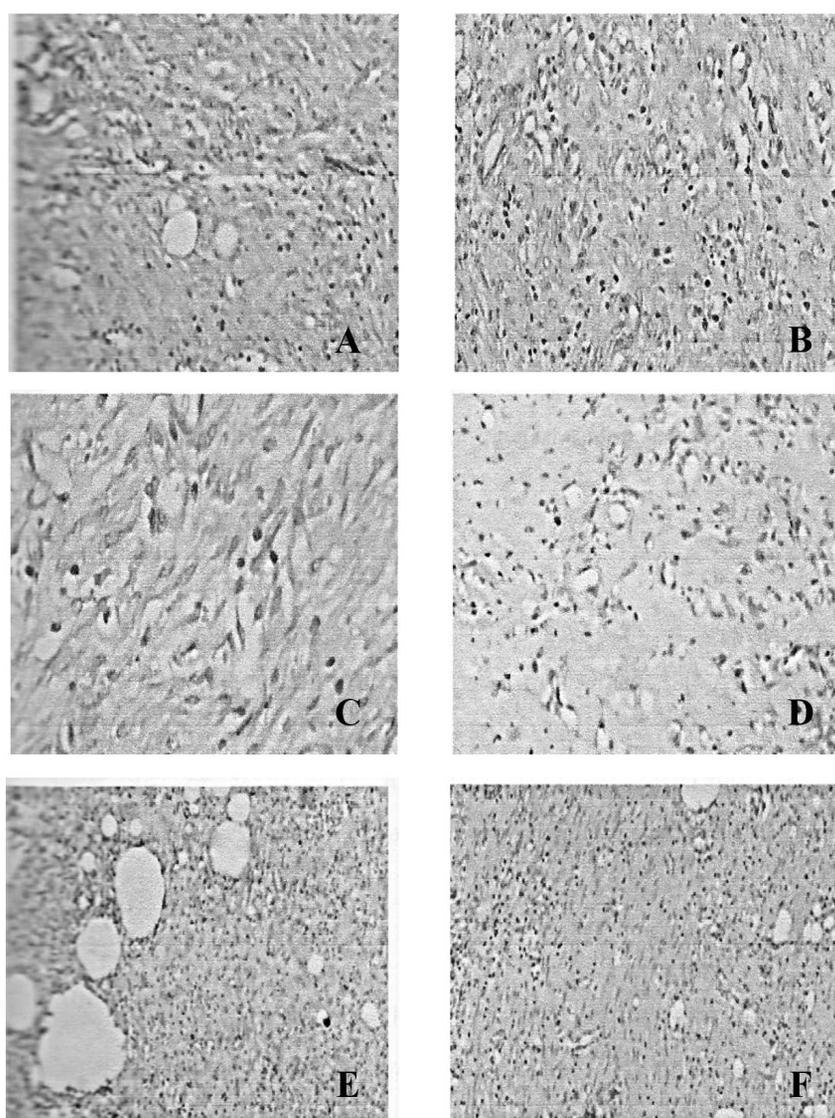


Figure 1 (A–F). Histopathological changes in granulation tissue of the rat skin excisional wound model. **A**: control animals (treated with simple ointment) showing poor collagenization, fibroblasts and poor neovascularization. **B**: animals treated with standard 5% povidone-iodine ointment topically, showing good collagen deposition. **C**: animals treated with 5% leaves extract ointment topically and **D**: animals treated with 10% leaves extract ointment topically, showing moderate level of collagen and fibroblasts. **E**: animals treated with 5% root extract ointment topically, showing moderate collagen deposition and **F**: animals treated with 10% root extract ointment topically, showing better healing and higher collagen deposition

### Period for epithelization

Falling of scab, leaving no raw wound behind, was taken as the end point of complete epithelization and the days required for this were taken as period of epithelization.

### Determination of breaking strength of granulation tissue

A piece of granulation tissue was fixed between two forceps and its breaking strength was measured by a constant and continuous water flow technique (11).

### Estimation of hydroxyproline

The dry granulation tissue was digested using 6 M HCl and the neutral hydrolysate was used to estimate the content of hydroxyproline by the method of Neuman and Logan (12).

### Protein content

Protein content was estimated by Lowry et al. method (13).

### Histopathological studies

On 11th post wounding day, two animals were sacrificed by deep anesthesia. The wounds were excised, having a 5 mm margin of normal skin around the edges of the wound, and placed in 10% formalin for histopathological examination. After the tissue was processed, mid wound vertical sections of each specimen were cut and stained with hematoxylin and eosin. The specimens were assessed under light microscopy for the progression of new epithelium and the formation of collagen in the wound.

### Statistical analysis

All the values were expressed as the mean  $\pm$  SEM. The data obtained through careful observation were analyzed by one way ANOVA followed by Dunnetts multiple comparison test and p values of less than 0.05% were considered as significant.

## RESULTS

Ten percent ointment of aqueous extract of roots shows 98% wound healing activity ( $p < 0.01$ ) after 12 days of treatment, and 5% ointment of aqueous extract of leaves shows about 88% wound healing activity ( $p < 0.01$ ) after 12 days and about 98% ( $p < 0.01$ ) after 16 days of treatment, whereas 10% ointment of aqueous extract of leaves and 5% ointment of aqueous extract of roots show 92% ( $p < 0.01$ ) healing activity after 12 days of treatment and about

99% wound healing activity ( $p < 0.01$ ), respectively, shown after 16 days of treatment (Table 1).

There is a significant increase in tensile strength, hydroxyproline content and protein content in all test group animals ( $p < 0.01$ , Table 2). There is also a significant decrease in period of epithelization in standard and in all treated groups as compared to control, particularly there was a significant decrease ( $p < 0.01$ ) in 10% root group (13 days) as compared with control (22 days) (Table 2).

Histopathological examinations of granulation tissue show better healing with higher collagen deposition with 10% ointment of aqueous extract of root in comparison to other test groups (Fig. 1. A–F).

## DISCUSSION

The present study showed that 10% ointment of aqueous extract of *Coleus aromaticus* possesses significant wound healing activity as evidenced by the significant increase in rate of wound contraction, decreased period of epithelization and increase in tensile strength of tissues. Similarly, the hydroxyproline and protein contents were significantly increased. Histopathological studies of granulation tissue of the 10% ointment of aqueous extract of root treated animals showed significant increase in collagen deposition with macrophages as shown in Figure 1 (A–F) than other ointments. Measurement of hydroxyproline could be used as an index for collagen turnover. An increase in breaking strength of granulation tissue indicates the enhanced collagen maturation perhaps by increased cross-linking.

In the present study, the phytochemical investigations of aqueous extract of roots and leaves showed the presence of carbohydrates, sterols, glycosides and proteins. Beside this, leaves were reported to contain terpenoids like carvacol, thymol, eugenol, chavicol and ethyl salicylate. It also contains flavonoids like cirsimaritin and sitosterol D glucoside and high amount of vitamin C. Several phytoconstituents like terpenoids (5, 14, 15), flavonoids (16–21) and vitamin C were known to promote wound healing process due to their antioxidant and antimicrobial activity. In addition, triterpenoids were reported to possess an ability to increase the collagen content, which is one of the factors promoting wound healing.

Furthermore wound healing activity can be attributed to free radical scavenging activity of flavonoids and vitamin C. Both these classes of phytochemicals are known to reduce lipid peroxidation not only by preventing and slowing the onset of cell necrosis but also improving vascularity. Lipid per-

oxidation is an important process in several types of injuries like burns, infected wound, skin ulcer etc. Hence, any drug that inhibit lipid peroxidation is believed to increase the viability of collagen fibrils, which in turn results in an increase in the strength of collagen fibre by increasing the circulation, preventing the cell damage and promoting the DNA synthesis. Elsewhere, a number of studies have shown the potential of medicinal plants in wound healing and wound care (17, 22–27).

Therefore, it is suggested that the wound healing activity of aqueous extract of roots of *Coleus aromaticus* is related to the presence of flavonoids and vitamin C.

In conclusion, the results of this study showed that the aqueous extract of roots of *Coleus aromaticus* may accelerate wound healing by enhancing epithelization and collagen deposition. The present investigations also offer scientific evidence to the folkloric use of *Coleus aromaticus*.

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