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# Evaluation of Wound Healing Activity of Leaves of *Bambusa arundinace*

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**ABSTRACT:**
For thousands of years, medicinal plants have played an important role throughout the world in treating and preventing a variety of diseases and still now, the preference of herbal drugs is ever increasing. Tribal people still depend on medicinal plants and most of them have a general knowledge of medicinal plants which are used as first aid remedies to treat cough, cold, fever, headache, poisonous bites and some simple ailments. Selected medicinal plant *Bambusa arundinacea* is used traditionally in hyperlipidemia, rheumatism, arthritis, obesity, inflammation, wrinkles etc. The present review explores relevant information about the screening of leaves obtained from *Bambusa arundinacea* in its wound healing potential as per the basis of its antiinflammatory, antibacterial and antioxidant properties. In the present study, the ethanolic extract of leaves of *Bambusa arundinacea* showed significant wound healing activity in the excision and incision wound models in rats. The progression of wound healing (area reduction) is 86.60% by 10% ethanolic extract at day 12$^{th}$. The results of the 12$^{th}$ day indicate that there is significant increase in the hydroxyproline content in extract groups, in incision wound model as compared to the group treated with standard drug i.e. povidone iodine.
**Keywords:** *Wound healing, Bambusa arundinacea, Excision, Incision.*

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## INTRODUCTION

Wound is a clinical entity and is as old as mankind, often possesses problem in clinical practice. A lot of research has been envisaged to develop the better healing agents and it has been a challenging task to discover healing agents and keep up pace with problems encountered. Wound may be defined as a loss or breaking of cellular and anatomic or functional continuity of living tissue. Wound healing or wound repair is the body's natural process of regenerating dermal and epidermal tissue[1].
The process of wound healing occurs in four phase[2]

1. Coagulation which prevents blood loss
2. Inflammation and debridement of wound
3. Repair including cellular proliferation
4. Tissue remodelling and collagen deposition

The antiulcer effects of the plant were primarily discovered through the reverse pharmacology mode. Different species of *Bambusa* are distributed in Rajasthan, Gujarat, Maharashtra, Madhya pradesh and Karnataka states of India. A large grass grows upto 40 meters in height. Leaves are alternate, long, base chordate, lanceolate, acute, and very rough; plant flowers once in its lifetime. Flowers are seen in large branched panicles, bisexual, small yellowish; fruits are oblong grains, grooved at one side. Stems are numerous, nodes prominent, internodes up to 50 cm long, greenish or yellowish. Very rarely some edible substance called Bamboo manna can be seen inside the hollow stems[3].

## MATERIAL AND METHODS

### Collection of plant, Drying and Authentification

Leaves of plant were collected from lands of distt Panna, Madhya Pradesh. They were dried and crushed. Authentification of plant was done by the Head of Botany department, SAFIA Science College, Bhopal (M.P).

### Extraction procedure

125 gm of powdered Leaves of *Bambusa arundinacea* was assembled in soxhlet apparatus with 250 ml of ethanol for 3 days in 6-6 hrs processing[4].

**Phytochemical screening**

In order to detect the various constituents present in the ethanolic extract of leaves of *Bambusa arundinacea*, phytochemical screening was proceeded to detect the presence of alkaloids, glycosides, carbohydrates, tannins, resins, flavonoids, steroids, proteins and amino acids[5].

**Excision wound model**

Animals were divided into four groups of six rats each and kept in separate cages. Group A (not treated) served as control, group B treated with povidone iodine and group C & group D with extract of leaves of *Bambusa arundinacea* 5% and 10% respectively once a day topically. The rats were anesthetized by administering ketamine (80 mg/kg i.p.). A full thickness of the excision wound of circular area was made on the shaved back of the rats 30 min later the administration of ketamine injection. The wounding day was considered as day 0. The wounds were treated with topical application of the ointments as described above till the wounds were completely healed. The wounds were monitored and the area of wound was measured on 3, 6, 9, 12, 15, 18, 21 post-wounding days and the mean % wound closure was reported. The period of epithelization was calculated as the number of days required for falling of the dead tissue remnants without any residual raw wound[6,7].

% of wound closure =

$$\frac{\text{Wound area on day 0 - wound area on day n}}{\text{wound area on day 0}} \times 100$$

where n = number of days 3$^{rd}$ , 6$^{th}$ , 9$^{th}$,12$^{th}$ ,15$^{th}$ and 18$^{th}$ day.

**Incision wound model**

The animals were divided into four groups of six rats each and kept in separate cages. Group A, serving as control, received only ointment base, group B received containing ointment povidone iodine, group C received 5% ethanolic extract of Bambusa arundinacea ointment while group D received 10% ethanolic extract of Bambusa arundinacea ointment daily for 10 days. The rats were anesthetized by administering ketamine (80 mg/kg. i.p.). Incision wounds of about 6 cm in length and 2 mm in depth were made with sterile scalpel on the shaved back of the rats 30 min after the administration of ketamine injection. The parted skin was kept together and stitched with black silk at 0.5 cm interval. Surgical thread (no. 000) and a curved needle (no. 9) were used for stitching. The continuous thread on both wound edges were tightened for good closure of the wound. The wounds of animals in the different groups were treated with topical application of the ointments as described above, for the period of 10 days. The wounding day was considered as day 0. The stitches were removed and the tensile strength of the wounds was determined on 10th day[8.9.10].

**Measurement of % wound contraction area**

The progressive changes in wound area were monitored by a camera (NIKON, Japan) every other day. Later on, wound area was evaluated by using tracing paper and by using simple algorithmic formula, wound area was calculated[11].

**Measurement of Epithelization time**

The epithelization time i.e. time required to develop a new epithelial layer at the wound site, was measured during the post wounding days with simultaneous application of various ointments at different groups[12, 13].

**Hydroxyproline ($C_5H_9O_3N$) Estimation**

The measurement of hydroxyproline can be used as an index for collagen turnover. Increase in hydroxyproline content indicates increased collagen synthesis, which in turn leads to enhanced wound healing. This assay was performed according to Shivhare et al. (2009) with small modifications[14].

**Procedure**[15, 16]

1. On the day of examination (6$^{th}$, 9$^{th}$ & 12$^{th}$) the animals were anaesthesized.
2. For the determination of hydroxyproline content, the wound tissues were excised and dried in a hot air oven at 60–70ºC to constant weight.
3. They were hydrolysed in 6 N HCl at 130ºC for 4 h in sealed glass tubes. The hydrolysate was neutralized to pH 7.0 and was subjected to Chloramine-T oxidation for 20 min.
4. The reaction was terminated by the addition of 0.4 M perchloric acid and color was developed with the help of Ehrlich reagent at 60ºC.
5. The absorbance was measured at 557 nm using a spectrophotometer (Shimadzu 1700, Pharmaspect, Japan). A standard curve was prepared using various dilutions of a 1 mg/ml stock solution of hydroxyproline with final concentrations ranging from 10 to 100 µg/ml and then values of skin tissue absorbance are matched and expressed as µg/mg skin.

**Tensile strength measurement**

Tensile strength of wound represents the effectiveness of wound healing. Usually wound-healing agents promote the gaining of tensile strength. Tensile strength (the force required to open the healing skin) is used to measure the completeness healing. On the 10th day after wounding, the sutures were removed and the tensile strength was measured. For measuring the tensile strength the rats were again anaesthesized and each rat was placed on a stack of towels on the middle of the board of tensiometer. Alligator clamp was tied on a longer fishing line with 1-l polyethylene bottle on the other end. The position of the board was adjusted so that the bottle receives a rapid and constant rate of water from a large reservoir, until the wound began to

open. The amount of water in the polyethylene bag was weighed and equated as the tensile strength of the wound. The tensile strength induced by the extract's ointment and by povidone iodine ointment treated wounds were compared with the control. The tensile strength was then taken to be the load in grams required to disrupt the wound sealing[17, 18].

## RESULT AND DISCUSSION

### Phytochemical findings

Ethanolic extract showed the presence of various primary and secondary metabolites, those were carbohydrates, resins, flavanoids, steroids, tannins. So it was assumed that ethanolic extract of *Bambusa arundinacea* having potential to heal the wounds, on the basis of their findings, responsible for anti-inflammatory, antioxidant and antimicrobial activities (previously studied)[19].

### Formulation development and evaluation

Suitable, smooth, non irritating, easily spreadable ointment was prepared taking both extracts and parameters such as pH (5.4 ± 0.6), spreadibility (49 ± 3.4 gm×cm/sec.), physical evaluation (colour, odour, grittiness, etc.), irritancy, etc. were evaluated[20]. The results were shown in table 1.

**Table 1**: Evaluation of ointment

| S. No | PARAMETER | OBSERVATION |
|---|---|---|
| 1. | pH | 5.4 ± 0.6 |
| 2. | Physical evaluation<br>Colour<br>Odour<br>Grittiness | <br>Greenish brown<br>Agreeable<br>No grittiness |
| 3. | Spreadibility | 49 ± 3.4 (gm×cm/sec.) |
| 4. | Irritancy | No irritancy |

**Effect on wound area reduction**: Day to day, wound area created in each rat of each group, was monitored and % reduction in wound area was calculated on 3rd, 6th, 9th, 12th, 15th and 18th post wounding day. Results were quite satisfactory as 100% reduction in wound area was achieved at 16th day in group D (final wound area in mm² 1.00 ± 0.004, initial wound area in mm² 427.6 ± 37.70) when ointment prepared by 10% ethanolic extract was applied to each animal 5% ethanolic extract group (group C) showed 100% reduction at 18th post wounding day (initial wound area in mm² 369.30 ± 12.65, final wound area in mm² 0.01 ± 0.00) as compared to control group (group A) where 100% reduction was achieved at 18th day (initial wound area in mm² 401.01 ± 23.32) and standard group (group B), in which povidone iodine ointment was applied to each animal in corresponding group and 100% reduction in wound area was achieved at 14th day after wound creation (initial wound area in mm² 351.30 ± 43.22 and final area in mm² 1.01 ± 0.65 ). All results were compared with one way anova test (cross matched by Dunnett's test) and showed respective $*P<0.050$. The results were shown in table 2, 3.

**Effect on Day of Epithelization**: New skin appeared in each wounded rat after continuous application of standard ointments and prepared test ointments. Control group showed after 17 ± 1.003 days, 5% ethanolic group showed after 15 ± 0.433 days, 10% ethanolic group showed after 14 ± 0.005 days whereas standard povidone iodine ointment group showed at 12 ± 0.340 days after the wounding day as was considered as day 0. Results were compared by Dunnett's test of one way anova; difference between control to test groups P= <0.001 (Dunnett's) standard to test groups P = <0.001 (Dunnett's).

**Effect on Hydroxyproline content:** As hydroxyproline is a newly formed collagen on the wounding site and it was evaluated by comparing amounts obtained through standard curve of hydroxyproline. Healed skins of wounded rats were taken at 6th, 9th and 12th post wounding day and then subjected to various serial wise tests to obtain the corresponding concentration on UV spectrophotometer. On 6th day, control, standard, 10% acetone and 10% ethanolic extract ointment group showed 14.55 ± 1.29, 21.49 ± 1.04, 18.08 ± 1.08 and 19.99 ± 2.36 µg of hydroxyproline respectively. On 9th day control group, standard ointment group, 10% acetone extract ointment group and 10% ethanolic extract ointment group showed 16.62 ± 1.45, 26.20 ± 1.76, 22.67 ± 0.55 and 24.46 ± 0.98 µg of hydroxy proline respectively, whereas on 12th day control, standard, 10% acetone and 10% ethanolic extract ointment group showed 19.44 ± 0.87, 33.06 ± 0.89, 25.90 ± 3.56 and 31.71 ± 1.56 µg of hydroxy proline respectively. Results were compared by Dunnett's test of one way anova; difference between control to test groups $*P<0.050$ (Dunnett's) standard to test groups $*P<0.050$ (Dunnett's).

**Effect on Increased Tensile Strength:** Tensile strength measured in postliminary pharmacological study, and standard group, 5% and 10% ethanolic extracts were used in a form of an ointment. On 10th day, in incision wound model, the tensile strength of healed incisions cuts were estimated and results were quite satisfactory, control group showed tensile strength of 414.06 ± 11.532, standard group showed tensile strength of 414.06 ± 11.532, group C, where ointment prepared with 5% ethanolic extract was applied to each volunteer in such group, showed tensile strength of 381.22 ± 10.692 group D, where ointment prepared with 10% ethanolic extract was applied to each volunteer in such group, showed tensile strength of 414.06 ± 11.532 gm×cm per sec respectively. When compared with one way anova test with additionally Dunnett's test. P value was obtained as < 0.001. Details are shown in table 5.

**Table 2**: % of wound area reduction

| Groups | Post wounding days (wound area in mm² ) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 3 | 6 | 9 | 12 | 14 | 16 | 18 |
| Group A (control) | 401.01 ± 23.32 | 298.66 ± 43.30 | 196.03 ± 43.32 | 89.20 ± 12.22 | 43.32 ± 11.65 | 27.34 ± 2.308 | 7.33± 2.43 | 0 |
| Group B (standard) | 351.30 ± 43.22* | 231.66 ± 39.65 | 143.40 ± 8.77* | 73.23 ± 9.45* | 19.21± 3.65* | 0.01 ± 0.040* | 0 | 0 |
| Group C (10% acetone extract ointment) | 369.30 ± 12.65* | 299.60 ± 19.99 | 203.00 ± 2.65* | 101.23 ± 7.55* | 39.70 ± 5.98 | 24.44 ± 2.766 | 3.23 ± 0.087* | 0 |
| Group D (10% ethanolic extract ointment) | 427.60 ± 37.70* | 341.30 ± 87.76 | 271.40 ± 1.65* | 134.32 ± 14.87* | 43.33 ± 3.408* | 12.14 ± 3.334* | 0 | 0 |

*n* = 6 albino rats per group, tabular value represents mean ±S.D.
(Comparison of A with B, C & D) **P*<0.050

**Table 3**: % Wound contraction area

| Groups | Post wounding days | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 3 | 6 | 9 | 12 | 14 | 16 | 18 |
| Group A (control) | 0.00 | 20.04 ± 0.24 | 47.20 ± 0.56 | 64.20 ± 0.44 | 83.00 ± 0.65 | 93.10 ± 1.77 | 99.20 ± 6.44 | 100 |
| Group B (standard) | 0.00 | 31.34 ± 0.45* | 49.30 ± 0.21* | 68.40 ± 4.55* | 89.80 ± 2.44* | 100 ± 1.22* | - | - |
| Group C(10% acetone extract ointment) | 0.00 | 20.67 ± 0.06 | 44.00 ± 1.94* | 65.60 ± 1.31 | 84.80 ± 1.43 | 97.60 ± 1.83* | 99.80 ± 7.77 | 100 |
| Group D (10% ethanolic extract ointment) | 0.00 | 23.20 ± 0.98* | 39.60 ± 1.05* | 67.20 ± 1.98* | 86.60 ± 2.45* | 99.30 ± 0.54* | 100 | - |

n= 6 albino rats per groups, tabular value represents mean ±S.D
(Comparison of A with B, C & D) **P*<0.050

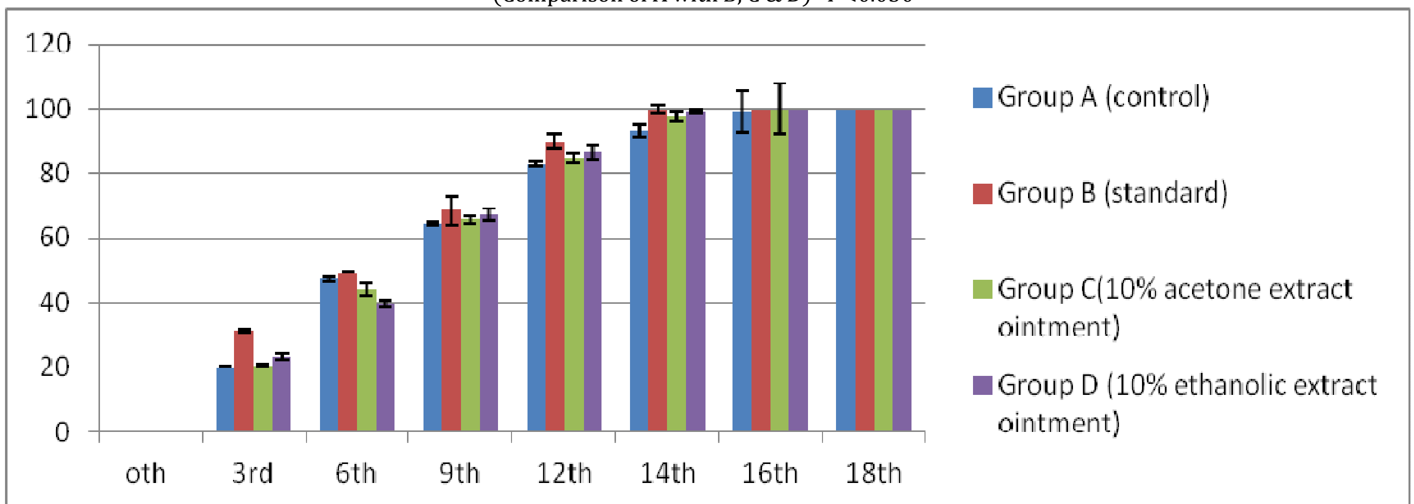


Fig 1: Comparison of % wound reduction area of all the groups

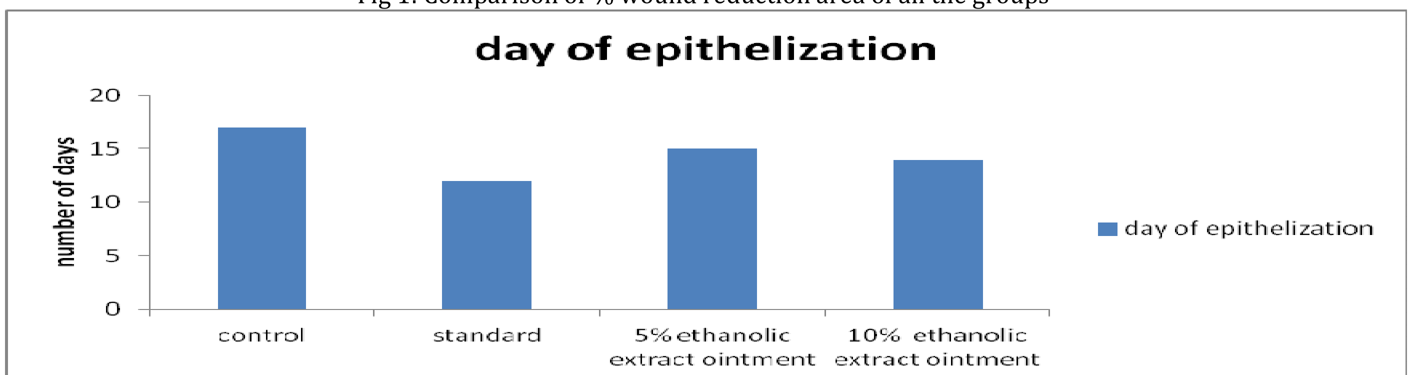


**Fig 2:** Comparison of A with B, C & D on day of epithelization

**Table 4**: Effect of ointment on Hydroxyproline content

| S. No. | | Day 6th | Day 9th | Day 12th |
|---|---|---|---|---|
| **Group** A | Control | 14.55 ± 1.29 | 16.62 ± 1.45 | 19.44 ± 0.87 |
| **Group** B | Standard | 21.49 ± 1.04* | 26.20 ± 1.76* | 33.06 ± 0.89* |
| **Group** C | Ethanolic extract 5% Ointment | 18.08 ± 1.08 | 22.67 ± 0.55 | 25.90 ± 3.56 |
| **Group** D | Ethanolic extract 10% Ointment | 19.99 ± 2.36* | 24.46 ± 0.98* | 31.71 ± 1.56* |

*n* = 6 albino rats per group, value represents mean ±S.D.
(Comparison of A with B, C & D) *$P<0.050$

**Table 5:** Tensile strength of healed wound, in different groups on 10th day

| **Content** | **Group A** | **Group B** | **Group C** | **Group D** |
|---|---|---|---|---|
| | **Control** | **Standard** | **5% Ethanolic extract ointment** | **10% Ethanolic extract ointment** |
| Mean ± S.D. | 256.73 ± 4.041g | 502.31 ± 41.016* g | 381.22 ±10.692*g | 414.06±11.532* g |

n=6; values are in mean ± S.D. All Pairwise Multiple Comparison Procedures (Tukey Test)
*$P<0.001$(Comparison of A with B, C & D)

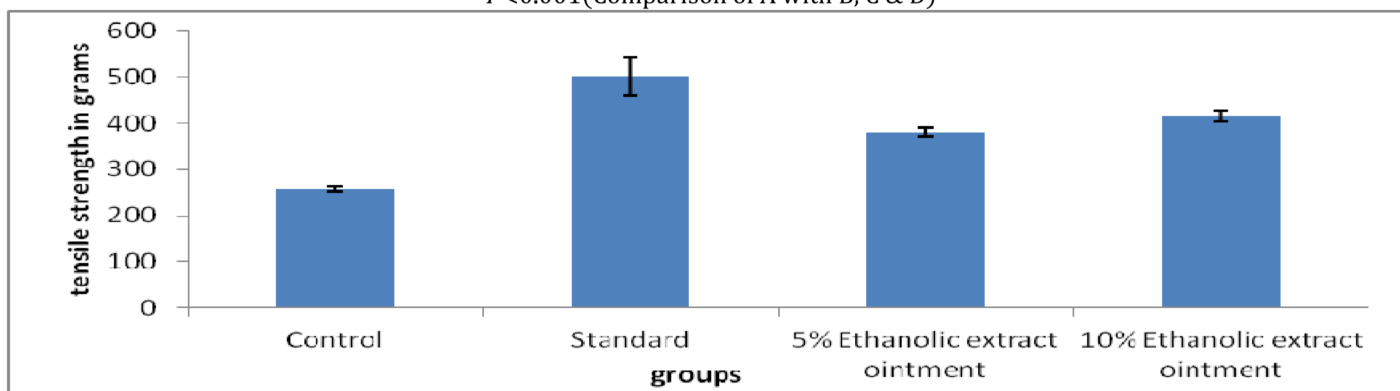


Fig 3: Tensile strength of healed wound, in different groups on 10th day

**CONCLUSION**

India has a rich traditional of plant based knowledge on healthcare. Tribal's and folklore traditions in India for treatment of cuts, wound and burns equally use a large number of plants/plant extracts/decoctions of pastes. In evaluation of wound healing activity of the plant *Bambusa arundinacea*, study was carried out through 2 models. First excision wound model and second incision wound model[21].

The results indicate that both (5% & 10%) ethanolic extracts of *Bambusa arundinacea* showed wound healing potential but 10% ethanolic extract ointment possesses a distinct prohealing stroke. This was demonstrated by a significant increase in the rate of wound contraction and by enhanced epithelization period. Phenolic contents were found to be present in ethanolic extract. Thus, the wound healing action of *Bambusa arundinacea* may probably be due to the flavanoids and steroids present in the plant. Hence, it can be concluded that *Bambusa arundinacea* has properties that render it capable for wound healing potential.

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