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# WOUND HEALING ACTIVITIY OF LEAF EXTRACT OF *WOODFORDIA FRUTICOSA* IN ALBINO RATS

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**ABSTRACT**

In Indian traditional system of medicine, *Woodfordia fruticosa kurz (Lytheraceae)* is widely used for its wound healing activity. The present study was aimed to evaluate its scientific validity. Here, we report the wound healing activities of hexane, methanol, ethanol, and aqueous extracts of *Woodfordia fruticosa* leaves in incision and excision wound models in rat. They were randomly divided into incision and excision wound groups. Each group was further subdivided into Control, Standard, HexE-treated, MeE-treated, EtE-treated, AqE-treated groups. The test substances were applied topically once daily. The tensile strength of the incision wound was measured on the seventh day after wound infliction. The wound area, epithelization period, percentage protection of excision wound was measured on the 1st, 4th, 7th, 10th, 13th, and 16th days. On the seventh day after wound infliction, the tensile strength of incision wound in all extract-treated groups was significantly higher than that of the control group. A significant increase of percentage protection was observed in all extract-treated groups (69.32%, 78.12%, 88.12%, and 62.1% for HexE, MeE, EtE and AqE, respectively).All types of extracts (HexE, MeE, EtE and AqE) exerted wound healing activity, but Ethanolic extract seemed to be the most active extract for wound healing.

**Key words:** *Woodfordia Fruticosa*, Wound healing activity, Incision and excision wound models.

## INTRODUCTION

A wound may be defined as a break in the epithelial integrity of the skin and loss of cellular and anatomic or functional continuity of living tissue (Ramzi SC *et al*., 1996).

Wound healing involves continuous cell–cell and cell–matrix interactions that allow the process involves platelet aggregation and blood clotting, formation of fibrin, an inflammatory response to injury, alteration in the ground substances, angiogenesis and re-epithelialization. Healing is not complete until the disrupted surfaces are firmly knit by collagen (Martin AA 1994, and Buffoni F *et al*., 1993) The basic principle of optimal wound healing is to minimize tissue damage and provide adequate tissue perfusion and oxygenation, proper nutrition and to restore the anatomical continuity and function of the affected part (Buffoni F *et al*., 1993) Antimicrobial ointments are used to reduce the risk of infection in minor cuts and burns. However, these have severe complications, partially effective in wound healing and involving high costs for therapy. Hence, the research for ideal drugs still continues and has been extended to herbal drugs.Several plants and herbs have been used experimentally to treat skin disorders, including wound injuries, in traditional medicine (Lodhi S *et al*., 2006 and Majumdar M *et al*., 2007).

*Woodfordia fruticosa*, a member of *Lytheraceae* family, is a much branched shrub with fluted stems and long, spreading branches, grows to a maximum height of 7 m, and occurs throughout India, ascending to an altitude of about 1,500 m in the Himalayas (Chadha YR, 1976). The plant bears numerous flowers, brilliant red in dense axillary paniculate-cymose clusters. The flowers yield a red dye and are employed throughout India for dying fabrics. The medicinal values of the plant and its parts

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indicate that the leaves of *Woodfordia fruticosa* possess antibiotic activity in vitro against Micrococcus pyogens var. aureus as well as sedative properties (Kritikar KR, 1999) and are reported to be used as a folk medicine in India and Nepal. Methanol and water extracts of the leaves of this plant inhibits DNA topoisomerase II (Dhar M L *et al*., 1968). In this present study, we under have taken the study of the wound healing effect of different extracts of *Woodfordia fruticosa*leaves.

## MATERIAL AND METHODS

### Plant material

The leaves and flowers of the plant were collected in the month of February, 2010 from Narsapur Forest, Medak. The plant was identified and authenticated. A Herbarium specimen of the plant was preserved in the Department of Pharmacognosy of our institute for further reference.

### Preparation of extracts

The leaves of the plant were shade dried, powdered mechanically and subjected to soxhlet extraction successively with hexane, methanol, ethanol and distilled water. After extraction the filtrate was concentrated on a rotary evaporator under vacuum at 20°C till a residual mass was obtained. Tween 20® (polyoxyethylene (20) sorbitan monolaurate) as a 10% solution in distilled water was used as vehicle for preparation of a 10% (w/v) of each extract for topical application. Ten grams of the dried extract was triturated with 9 mL of vehicle. Vehicle was then added gradually until the final concentration of the extract was 10%.

### Experimental animals

Healthy male Wister albino rats at 8 weeks of age were purchased from Ghosh Enterprises, Kolkata. They were housed in a room, maintained at approximately 25 ± 2 ˚C. The photo period was 12 hrs light and 12hrs dark cycle. For feeding, the rodent laboratory diet used which is supplied from Ryan biotechnology Pvt Ltd. Hyderabad. Diet contains 22.28% of protein, 5.58% of total oil, 3.86% of dietary fibres, 9.12% of moisture, 0.13% of salt, 3.69% of ash, and 55.44% of carbohydrates.

The animals were randomly divided into incision and excision groups. Each group was further divided into six subgroups composed of six animals per subgroup:

1. Control group (treated with Tween 20®)
2. Standard group, (treated with 0.2%w/w nitrofurazone ointment)
3. HexE-treated group.
4. MeE-treated group.
5. EtE-treated group.
6. AqE-treated group.

### Effect of *W. fruicosa* extracts on healing of incision wounds

The effect of *W. fruticosa* extracts on the healing of incision wounds was investigated by using the model of Baie and Shiekh. The animals were anesthetized intraperitoneally with sodium pentobarbital at 60 mg/kg BW during induction of the wound. The right side on the back of each animal was shaved and depilated (Fem hair remover). Next, a 3 cm long, midline incision was made through the skin with a sharp scalpel and was closed with a 0.5 cm spaced interrupted sutures with black silk no.3-0 to secure the edges. After creating the wound, 0.5 mL of the test substance was topically applied to the affected area once daily. On the seventh day after inflicting the wound, the animals were sacrificed with intra-peritoneal injection of sodium pentobarbital at 100 mg/kg BW. Sutures were removed and tissues were isolated from the healed wound to assess its tensile strength, as described below.

### Assessment of the tensile strength of incision wounds

Healing of incision wounds was evaluated by measuring the tensile strength on day 7 after inflicting the wound. Sutures were removed and the skin tissue was cut 1 cm away from each side of the wound. The isolated wound tissues were used to measure the load (force) required to break the tissue with a computerized tensiometer (JZYW-200B Interface Tensiometer). Tensile strength was calculated using the following formula **(**Baie and Shiekh *et al*., 2000):

**Tensile strength (N/cm2) = breaking force (N)/area (cm2);**

Where,

Area (cm2) = thickness (cm) × width (cm)

### Effect of *W. fruicosa* extracts on healing of excision wounds

Animals were anaesthetized with light ether prior to and during creation of the wounds according to the method of Morton and Malone, 1972. The hairs on the skin of back surface of the animals were removed by wiping with a suitable depilatory (Fem hair removing cream) with the help of a cotton swab. A circular wound of about 500sq mm was made on depilated dorsal thoracic region of animals by cutting the skin of the animals by using forceps and scissors. The entire wound was left open. The observation of percentage wound closure was made on 1st, 4th, 7th, 10th, 13th and 16th days post wounding days. (The area of the wound was marked by placing a transparency sheet over the wound) (Kumara Swamy HM *et al*., 2007). The wound areas recorded were measured in square millimeter by using graph paper. This was taken as the initial wound area healing.

The simple ointment, standard drug and test drug was applied on wound everyday up to 16th day. The

wound area of each animal was measured on the 1st, 4th, 7th, 10th, 13th, and 16th days in square millimeter by using graph paper. They were observed thoroughly for epithelization and contraction of wound. The complete epithelization was observed by fall of scab without any raw wound area. Number of days required for falling of scab without any residual raw wound gave the period of epithelization (Anuradha R *et al*., 2008). The percentage protection was calculated on the 16th day by using the following formula and tabulated in table (Agrahari *AK et al*., 2010).

$$\%\ Protection = \frac{100 - (Final \times 100)}{Initial}$$

## STATISTICAL ANALYSIS

The results are presented as mean ±SEM (standard error of mean) and subjected to "One-way ANOVA" followed by Dunkens multiple range test. The values of $p<0.05$ were considered significant.

## RESULTS

### Effect of *W.fruticosa* extracts on healing of incision wounds

As shown in Table 1, the tensile strength of control was 13.42 ±0.92 N/cm2. A significant increase of tensile strength was observed in all extract-treated groups (15.32 ±0.56, 18.76 ±1.73,20.56 ±0.86, and 4.63 ±0.86 N/cm2 for HexE, MeE, EtE and AqE, respectively) compared to the vehicle-treated group. Tensile strength of incision wounds was significantly increased in the ethanolic extract treated group, as compared to control and extract treated groups. Its also showed significant wound healing activity, which was comparable with standard drug nitrofurazone.

**Table 1. Tensile strength of incision wounds treated with extracts of *W.fruticosa* on day 7 after wound infliction**

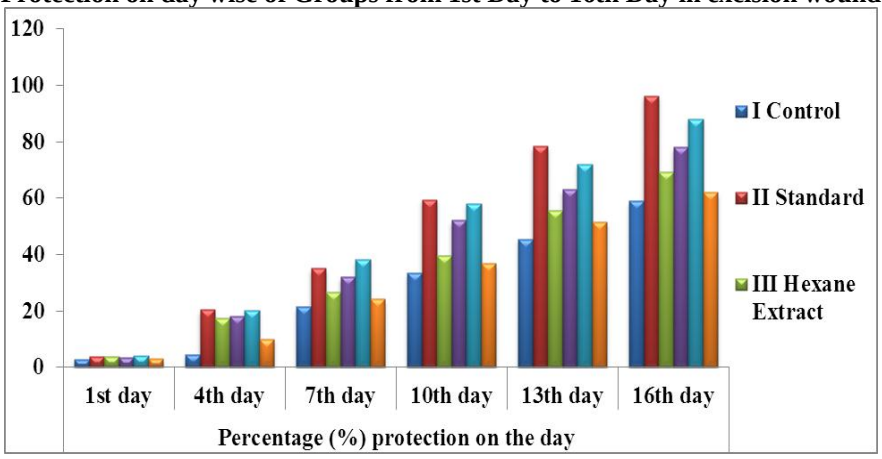
| Treatment group | Tensile strength (N/cm2) |
|---|---|
| Control | 13.42 ± 0.92 |
| Standard | 21.79 ±1.42 |
| Hexane extract | 15.32 ±0.56 |
| Methanol extract | 18.76 ±1.73 |
| Ethanol extract | 20.56 ±0.86 |
| Aqueous extract | 4.63 ±0.86 |

Values are presented as mean ± S.E.M; n = 6; *P<0.05 and **P<0.01 vs. Tween 20®.

**Table 2. Percentage of Protection on day wise of Groups from 1st Day to 16th Day in excision wound model**

| Group | Treatment | Percentage (%) protection on the day | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1st day | 4th day | 7th day | 10th day | 13th day | 16th day |
| **I** | **Control** | 2.91 | 4.68 | 21.56 | 33.41 | 45.61 | 58.95 |
| **II** | **Standard** | 3.91 | 20.48 | 35.12 | 59.61 | 78.42 | 96.43 |
| **III** | **Hexane Extract** | 3.89 | 17.36 | 26.67 | 39.68 | 55.67 | 69.32 |
| **IV** | **Methanol Extract** | 3.64 | 18.14 | 32.12 | 52.17 | 63.14 | 78.12 |
| **V** | **Ethanol Extract** | 4.12 | 20.36 | 38.15 | 58.16 | 72.16 | 88.12 |
| **VI** | **Aqeous Extract** | 3.11 | 10.12 | 24.32 | 36.89 | 51.61 | 62.10 |

**Fig 1. Percentage of Protection on day wise of Groups from 1st Day to 16th Day in excision wound model**



**Effect of *W.fruticosa* extracts on healing of excision wounds**

It was found that all the four groups showed decreasing of wound area from day to day. Wound healing contracting ability was significantly greater than that of the control group in all extract-treated groups. As shown in Table 2, the control Group showed 58.95% protection. A significant increase of percentage protection was observed in all extract-treated groups (69.32%, 78.12%, 88.12%, and 62.1% for HexE, MeE, EtE and AqE, respectively). Treatment with ethanolic extract of leaves showed significant ($p<0.05$ and $p<0.001$) dose-dependently decrease in epithelization period, when compared to control and extract treated groups. Faster wound healing was observed at day 13th and 16th day in rats treated with ethanolic extract, which is closer to that of standard Nitrofurazone indicating significant wound healing activity.

## DISCUSSION

Traditionally, medicinal plants have been used for many years as topical and internal preparations to promote wound repair. Current researches are devoted to validating their efficacy and to uncover the mechanisms responsible for this activity. 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 (Nguyen DT*et al*., 2009)

This study demonstrated that four different extracts of *W.fruticosa* were able to increase the rate of wound healing for both incision and excision wounds. Seven days after inflicting the wound, the tensile strengths in the HexE, MeE, EtE and AqE-treated groups were significantly higher than that in the vehicle-treated group. The increased tensile strengths observed as a characteristic of the healing activity of extracts of *W.fruticosa* may indicate an increase in collagen in the wound lesion. The present findings agree well with previous studies showing an increase of tensile strength or collagen synthesis in wounds treated by ethanolic extracts of flowers (Neeraj V *et al*., 2013).

The complex process of healing involves various phenomena like wound contraction, granuloma formation and collagenation etc. Wound contraction plays a significant role in healing of excision wound. Collagen is the predominant extracellular protein in the granulation tissue of a healing wound and there is a rapid increase in the synthesis of this protein in the wound area soon after an injury, which provides strength and integrity to tissue matrix (Reddy J S*et al*., 2002). Measurement of the hydroxyproline, which comes from the breakdown of collagen, has been used as an index of collagen turn over (Agarwal PK *et al*., 2009). In the present study, ethanolic extract of leaves showed wound healing and increase in tensile strength of incision wounds. It also increased the wet and dry weight and the hydroxyproline content of granulation tissue, indicating the presence of higher collagen content and its turnover, leading to rapid healing. In wound healing process, collagen formation peaks at day 7 and epithelialization occurs in 48 h under optimal conditions (Garber AM, 2002). The present results also indicated significant decrease in wound area from day 7 onwards, indicating early healing in excision wound model.

## CONCLUSION

The present study demonstrates the wound healing effects of *W.fruticosa* leaf extracts for both incision and excision wounds. All types of extracts (HexE, MeE, EtE and AqE) exerted wound healing activity, but ethanolic extract was the most active extract for wound healing.

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