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# Evaluation of Antibacterial and Wound Healing Activities of Ethanolic Extract of *Linum Usitatissimum* (Flax Seed) on Rabbits

**Ravishankar Kakaraparthy, K. Sruthi, Monika Banerjee***

Department of Pharmacology, Aditya College of Pharmacy, Surampalem, Andhra Pradesh, India-533437

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**Corresponding Author:**
**Ms. Monika Banerjee**
Department of Pharmacology
Aditya College of Pharmacy,
Surampalem, Andhra Pradesh, India.
**Email:** monikabanerjee2232@gmail.com

**ABSTRACT**

**Aim:** *Linum usitatissimum* was evaluated for its antibacterial and wound-healing capacity.

**Methods:** This method involved the collection and authentication of the plant, preparation of herbal extract, phytochemical analysis for the prepared extract, development of an herbal formulation for the prepared extract, evaluation of the antibacterial activity of *Linum usitatissimum* seed extract and the evaluation of the wound healing activity by Excision wound model. The antibiotic activity was studied by cup plate method using a nutrient agar medium. The zone of inhibition was then determined after culturing and incubation.

**Results:** The results of the extract of *Linum usitatissimum* show antibacterial activity as demonstrated by zone of inhibition when cultured with selected bacterial species (both gram positive and gram negative). Different concentrations of (200 µg /ml) and (400µg/ml) were used. The extract showed an improved wound healing capacity from the third application as demonstrated by the increase in the percentage of healing (9.55 ± 0.04%) with a significant increase on the sixth day (23 ± 0.4%). Betadine was used as a standard medicament for wound healing.

**Conclusion:** The present research study demonstrated that the medicinal plant *Linum usitatissimum* seed extract possesses significant antibacterial and wound-healing properties. Based on the observation and the significant results obtained, the researchers can draw that the ethanolic seed extract of *Linum usitatissimum* produced antibacterial and wound healing activities and the results were authenticated by comparing with reference drugs. Further scientific studies can be taken up and detailed study on the preliminary phytochemical constituent present which may be responsible for these biological activities. At this junction of time, this research study provides justification and scientific evidence for further pharmacological activities that are evaluated in our laboratory.



## Introduction

A wound is defined as harm or disruption to the typical anatomical structure and function. Injury to parenchymal organs, arteries, nerves, muscles, tendons, bones and even deeper subcutaneous tissue might result from this. Simply having an epithelial integrity break in the skin is another possibility [1]. Wounds may develop as a result of pathological processes that begin either outside or inside the involved organ. Every day attacks, including those that are physical, chemical and biological, as well as cell death and destruction, are bound to result in wounds. Wounds lead to disturbed tissue integrity and disruption of cellular-level biological activity in living tissues. In biological research, numerous investigations have been made to learn how to hasten wound healing. Recent years have seen a number of researchers narrow their attention to the traditional ways of promoting wound healing by using plant extracts, herbal powders and plant oils. Antiseptics are used to heal cuts, scratches, burns, grazes and other small injuries where the skin is broken as well as a preventative measure prior to piercing the skin (such as an injection). Wounds may develop as a result of pathological processes that begin either outside or inside the affected organ. They could result from illness processes or have another etiology, such as accidental, worldwide, or other. Physiological responses to the noxious factor include inflammation, hemorrhage and vascular constriction with coagulation, complement activation and bleeding.

In recent days many scientists worked that wound wound-healing properties of Flax seed [2, 3]. Ancient Egypt had considerable flax cultivation and mummies were embalmed with linen. The walls of the temples even included paintings of flax in bloom. The seeds and fiber of the flax plant are widely prized [4]. The seeds can be squeezed into oil and are edible. The seeds are oval, flattened, elongated, 4-6mm long and 2-3mm brown with a minutely pitted surface. Seeds are rounded at one end. The major constituents include polyunsaturated fatty acids (PUFA) of the omega-3 family [5], soluble dietary fibers, lignans, proteins and carbohydrates found in flax seed, all known for their particular biological activity and functional qualities [4]. However, it contains only trace amounts of harmful substances such as cyanogenic substances, protease inhibitors and cadmium. Many of the bioactive substances found in flax seeds are

advantageous to people in general. Compelling data from animal experiments support the health effects and their implications in reducing the risk of cardiovascular diseases, strokes, diabetes and cancer, but more human research is required [5]. Present article deals about the wound healing property of *Linum usitatissimum* in Antibacterial and wound healing properties of developed formula compared with the market leading benchmark samples.

## Materials and Methods

**Collection of plants:** Flaxseeds were purchased from an Andhra Pradesh agriculture seed store in Kakinada, Andhra Pradesh, India. The sample was thoroughly cleaned with tap water before being allowed to air dry in the shade. Then, using a mechanical grinder, they were ground into a coarse powder.

**Preparation of ethanolic extract of *Linum usitatissimum*:** The powder sample was macerated with ethanol for 3 days and then subjected to hot percolation (the process of continuous agitation on a rotator shaker for about 7-8 hours at 90rpm). The solution was further filtered through Whatman filter paper no. 42 (125 mm) separately. And the concentrated liquid was subjected to distillation at 80°C. The appearance of solvent was taken as the termination of extraction. The extract was then boiled in a water bath maintained at 50°C to obtain an ethanolic extract. The concentrated product was dried using desiccator with anhydrous calcium chloride. The extracts were then preserved in an airtight container under refrigeration for future use [6].

**Preparation of ointment for wound healing activity 10 %( w/w):** The ingredients of the formulation of absorption ointment base (50g) includes: Wool fat (2.5g); Hard paraffin (2.5g); Cetostearyl alcohol (l 2.5g) and White soft paraffin (42.5g) [7].

**Procedure:** Hard paraffin and cetostearyl alcohol were taken in a China dish kept in a water bath at 70ºC. Wool fat and white soft paraffin are added to this mixture and stirred until all the ingredients are melted. About 5g of the extract incorporated in 45g absorption ointment base was separately mixed with the above prepared simple ointment base. Transfer the ointment into a suitable container and labeled properly [8-15].

**Antibacterial Assay:** The seed extract of *Linum usitatissimum* plant extract was evaluated for antibacterial activity against several gram-positive and gram-negative organisms. The antibacterial activity of the ethanolic extract was determined using the agar cup-plate method. About 20ml of sterile nutrient agar medium was poured into a sterile Petri dish using the spread plate technique and allowed to solidify. Seeded with the organism by pour plate technique using sterile top agar (4ml) containing 1 ml culture. Bores were made in the medium using a sterile borer [6]. Dried ethanolic whole plant extract of Linum usitatissimum was dissolved in DMSO to obtain different concentrations (200 and 400 µg/ml) and 0.05 ml of different concentrations of the extract were added to the respective bores [9]. About 0.05ml of Gentamycin at a concentration of 25 µg/ml was taken as a standard reference. All the plates were kept in the refrigerator at 2 to 8°C for a period of 2 hours for effective diffusion of the test compounds and standard. Later, they were incubated at 37°C for 24 hours.

**Measuring the zone of inhibition:** The presence of a definite zone of inhibition of any size around the cup indicates antibacterial activity and was measured using graph paper or a scale [6].

**Wound healing activity**

**Experimental animal:** The experimentation was conducted on 1.8-2.5 kg rabbits of either sex. Each animal was kept in a separate plastic cage. They were given access to a set amount of food. Fresh veggies and unlimited water are added to a commercial pellet diet. The animal was kept in a holding area that had 12 hours cycles of light and darkness. The room was set to 23 ±2°C and had a 45% to 55% humidity level [10, 14, 16].

**Ethics Approval:** All the experimental protocols were approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) with registration number: 1269/PO/E/S/08/CPCSEA.

**Excision wound model:** The hair of the lower back and left flank of the test animals were fully shaved and cleaned. The desired area was locally sterilized and anesthetized with the subcutaneous injection of 2% lidocaine. A full thickness of the excision wound of a circular area of 500 $mm^2$ and 0.2 cm depth was created [11]. The wound was made by excision of the skin, within the border of the template to the level of loose

subcutaneous tissue, using a size no. 15 scalpel blades and forceps, the wound of the animal was divided into 2 groups.

Group 1 was treated topically with povidone iodine ointment (Betadine) as a standard healing agent [12]. Group 2 was treated with ethanolic extract of *Linum usitatissimum* with a dose concentration of 10% (w/w) ointment, where 10g of extract incorporate into 100g of simple ointment base respectively. Application of drug using a sterile swab, twice a daily test animal with infected wound was excluded from the study [18].

All ethical issues were considered in the surgical procedure and during the treatment, the area of the wound on the first day was considered as 100% and the wound areas on subsequent days were compared with the wound on the first day healing percentages on different days of treatments calculated between the initial wound surface areas and that on the day of measurement [12]. They were observed thoroughly for epithelization and concentration of wound. The number of days required for falling off the scab without any residual raw wound gave the period of epithelization. The percentage protection was calculated on the 18th day by using the following formula and tabulated [13, 16-19].

$$\text{Percentage protection} = \frac{\text{Wound area at 0 day} - \text{Wound area at day n}}{\text{Wound area at 0 day}} \times 100$$

## Statistical analysis

Mean and SEM were used to express the results. Dunnett's test was used to compare the treatment groups to the standard groups after one-way analysis of variance (ANOVA). The threshold for statistical significance in each test was set at $p<0.05$ (95% level) [13].

## Results

**Preliminary Phytochemical analysis:** Various chemical tests were carried out and tabulated below. Preliminary phytochemical tests revealed the presence of alkaloids, flavonoids, tannins, saponins, carbohydrates, proteins and glycosides.

**Table 1: Results for Preliminary phytochemical analysis of ethanolic extract of *Linum usitatissimum***

| Compounds | Chemical test | Observation |
|---|---|---|
| **Alkaloids** | Dragendruff's Test | Positive |
| | Hager's Test | Positive |
| | Wagner's Test | Positive |
| **Carbohydrates** | Molisch Test | Positive |
| | Benedict's Test | Positive |
| | Fehling's Test | Positive |
| **Flavonoids** | Lead acetate test | Positive |
| | Zn-HCL reduction test | Positive |
| | NaoH test | Positive |
| **Glycosides** | General tests | Positive |
| | Modified Bontrager's test | Positive |
| | Legals test | Positive |
| **Proteins** | Xanthoproteic test | Positive |
| | Millions Test | Positive |
| | Biuret test | Positive |
| **Saponins** | Foam test and Froth test | Positive |
| **Tannins** | Ferric chloride test | Positive |
| **Triterpenoids** | Salkowski test | Positive |
| | Liebermann-Buchard's test | Positive |

**Anti-Bacterial Activity:** The plant extract of *Linum usitatissimum* was studied for antibacterial activity employing the cup plate method. Bacteria used were *Bacillus Subtilis, Staphylococcus aureus, Escherichia coli, Staphylococcus werneri, Pseudomonas aeruginosa, Pseudomonas putida*. Both gram-positive and gram-negative bacteria were sensitive to the extract. The zone of inhibition recorded for various organisms as shown in the activity of the ethanolic extract of the seed was comparable to that of the reference standard drug Gentamycin cup (25µg/ml). *Linum usitatissimum* seed extract exhibited good antibacterial activity and results were tabulated.

**Table 2: Antibacterial activity of *Linum usitatissimum* seed extract**

| Type of Bacteria | Name of microorganism | *Linum Usitatissimum* (200µg/ml) | *Linum Usitatissimum* (400µg/ml) | Gentamycin (25µg/ml) |
|---|---|---|---|---|
| Gram-Positive Bacteria | *Staphylococcus aureus* | 12.5 (± 0.04) | 14 (± 0.07) | 21 (± 0.2) |
| | *Bacillus Subtilis* | 11.4 (± 0.1) | 12.7 (± 0.02) | 20 (± 0.8) |
| | *Staphylococcus werneri* | 11.2 (± 0.05) | 12.5 (± 0.04) | 19.6 (±0.07) |
| Gram-Negative Bacteria | *Escherichia coli* | 11.4 (± 0.05) | 13.2 (± 0.04) | 20 (±0.05) |
| | *Pseudomonas aeruginosa* | 11.7 (± 0.05) | 12.3 (± 0.04) | 19.9 (± 0.04) |
| | *Pseudomonas putida* | 11.13 (±0.04) | 13.8 (±0.02) | 20 (±0.05) |

***Values are expressed as mean ± SEM; n=3***

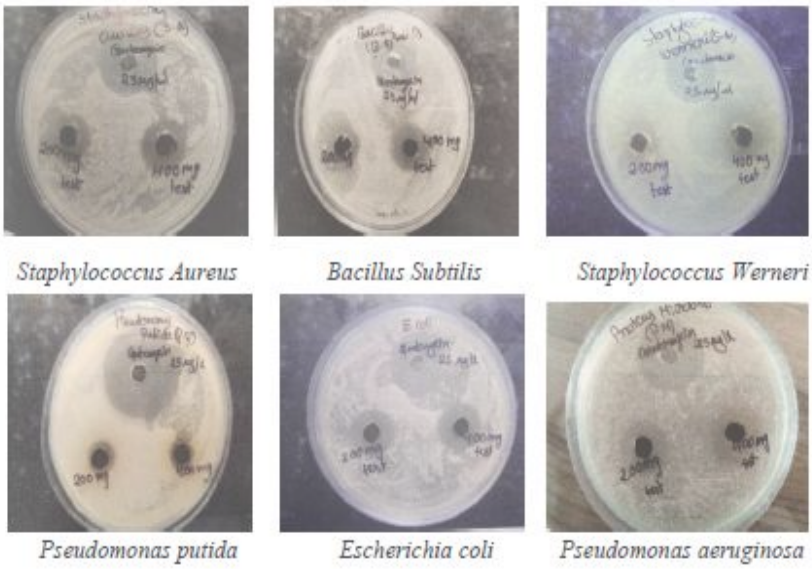
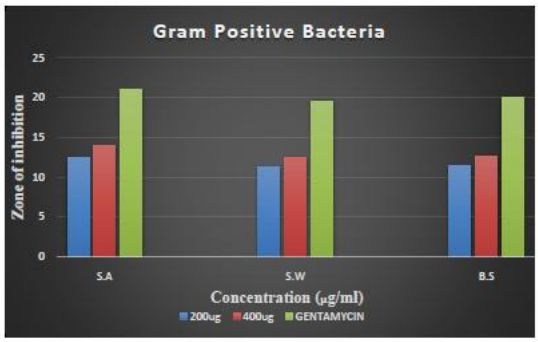
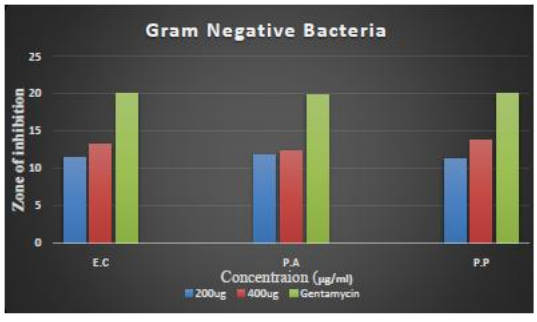



**Figure 1: Zone of inhibition of *Linum usitatissimum* seed extract Gram-positive and Gram-negative Bacteria**




*Staphylococcus aureus (S.A)*
*Bacillus Subtilis (B.S)*
*Staphylococcus werneri (S.W)*

**Figure 2a: Zone of inhibition of *Linum usitatissimum* seed extract of Gram-positive**




*Pseudomonas putida (P.P)*
*Escherichia coli (E.C)*
*Pseudomonasaeruginosa (P.A)*

**Figure 2a: Zone of inhibition of *Linum usitatissimum* seed extract of Gram-positive**

**Results of wound healing activity:** The wound healing contracting ability of ethanolic seed extract of *Linum Usitatissimum* in the excision wound model in the 10% extract ointment treated group showed significant wound healing from 3rd day onwards which was comparable to that of the standard drug i.e., Betadine treated group. The wound closer time was lesser, as well as the percentage of wound concentration was much more with dose extract and 10% *Linum Usitatissimum* was observed on (23± 0.47) 6thday, which was almost similar to that of Betadine treated group (36.3± 0.4) 6thday. The 10% extract animals showed significant wound healing from the 6th day onwards and achieved 100% wound closure on the 18th day as shown in the table. The result of the present study revealed that the concentration of ethanolic extract *Linum Usitatissimum* has significant wound healing activity in the excision model.

**Table 3: Percentage of wound healing shown by ethanolic extract of *Linum usitatissimum* seed extract in excision wound model**

| Treatment | Percentage of wound healing | | | | | |
|---|---|---|---|---|---|---|
| | 3rd day | 6th day | 9th day | 12th day | 15th day | 18th day |
| *Linum usitatissimum* | 9.55 (±0.04) | 23 (±0.47) | 52.3 (±0.5) | 80.9 (±0.2) | 85 (± 0.2) | 94.65 (±0.1) |
| **Standard (Betadine)** | 18.18 (±0.01) | 36.3 (±0.4) | 63.6 (±0.4) | 77.2 (±0.02) | 95.45 (±0.4) | - |

*Values expressed as mean ±SEM: n=2 animals*

**Table 4: Effect of topical application of Ethanolic Extract of *Linum Usitatissimum* seed extract of excision wound model**

| Treatment | | Post Wounding Days | | | | | |
|---|---|---|---|---|---|---|---|
| | 0th day | 3rd day | 6th day | 9th day | 12th day | 15th day | 18th day |
| *Linum usitatissimum* | 2.1 (±0.02) | 1.9 (±0.05) | 1.6 (±0.02) | 1 (±0.02) | 0.4 (±0.04) | 0.3 (±0.04) | 0.1 (±0.02) |
| **Standard (Betadine)** | 2.2 (±0.04) | 1.8 (±0.02) | 1.4 (±0.4) | 0.8 (±0.05) | 0.5 (±0.05) | 0.1 (±0.01) | - |

*Values expressed as mean ±SEM: n=2 animals*

## Discussion

The preliminary phytochemical screening of *Linum usitatissimum* was performed and the obtained results revealed the presence of alkaloids, flavonoids, phenolic compounds, tannins, and glycosides. These phytochemicals especially flavonoids and phenolic compounds could be attributed to anti-inflammatory antibacterial and wound-healing properties [20]. In this present research work, we observed that the ethanolic extract of *Linum usitatissimum* exhibits significant antibacterial activity showing a good zone of inhibition against several gram-positive and gram-negative strains. The result of antibacterial drug activity compared with the standard drug Gentamycin. The highest antibacterial activity was shown with a higher concentration of *Staphylococcus Werneri* (14±0.07mm) for the concentration of (400µg/ml). The least activity was shown with gram-negative bacteria *Pseudomonas aeruginosa* (12.3 ±0.04).

Further, our research study was evaluated for its wound-healing property and the medicinal plant *Linum usitatissimum* was evaluated using an excision model in albino rabbits. The prepared 10% extract with incorporated into a simple ointment and that ointment is applied to the wound regularly.

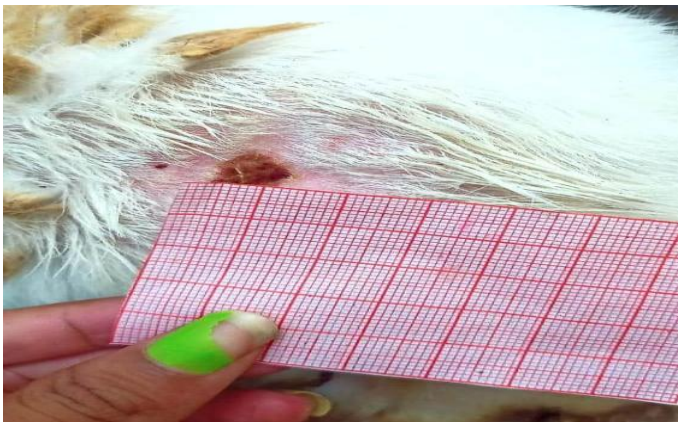
The results of the healing property were measured and the results were compared with standard antibacterial ointment 5% povidone-iodine (Betadine) ointment. The test extract ointment was applied to the wound and the epithelization was observed and it showed improved wound healing capacity from the third application demonstrated by an increase in the % of healing (9.55±0.04) % with a significant increase on the sixth day (23± 0.4) %. And found that the test extract ointment possessed decent and the authentic photograph was enclosed.

**0th Day**

**3rd Day**

**6th Day**

**9th Day**



**12th Day**

**15th Day**

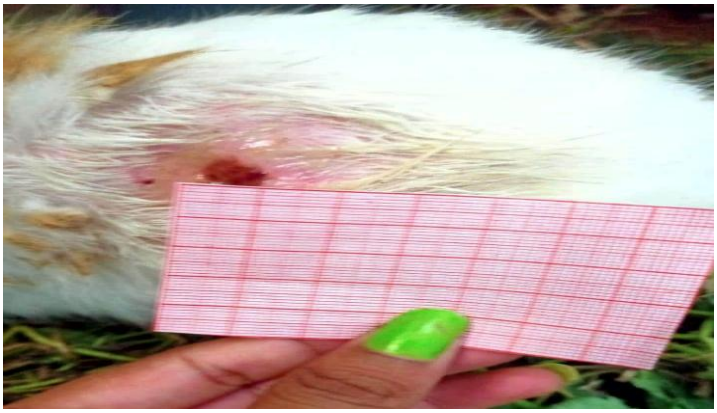
**18th Day**

**Figure 3: Effect of *Linum usitatissimum* 10% extracts ointment on wound (0th day to 18th day)**

0th Day

3rd Day

6th Day



12th Day

15th Day

**Figure 4: Effects of Betadine on wounds (0th Day to 15th Day)**

## Conclusion

The present research study demonstrated that the medicinal plant *Linum usitatissimum* seed extracts possesses significant antibacterial and wound-healing property. Based on the observation and the significant results obtained, the researchers can draw that the ethanolic seed extract of *Linum usitatissimum* produced antibacterial and wound healing activities and the results were authenticated by comparing with reference drugs. Further scientific studies can be taken up and detailed study on preliminary phytochemical constituents present which may be responsible for these biological activities. At this junction of time this research study provides justification and scientific evidence for further pharmacological activities that can be evaluated in our laboratory.

## Abbreviation

**PUFA:** Polyunsaturated fatty acids
**DMSO:** Dimethyl sulfoxide

## Conflict of Interest

The authors declare no conflict of interest.

## Acknowledgement

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