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ORIGINAL ARTICLE

# Exploring the Topical Gel of *Thespesia populnea* leaf Extract for *in vivo* Wound Healing Efficacy

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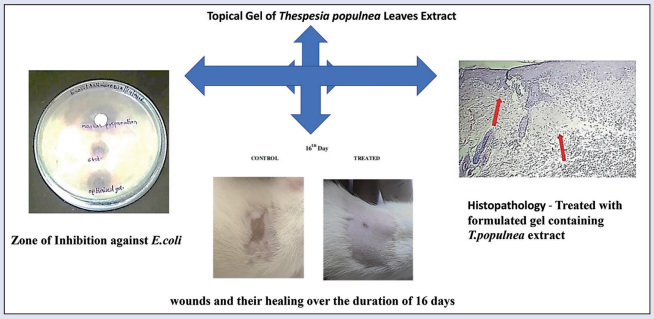
## ABSTRACT

**Background/Aim:** *Thespesia populnea* is a plant known for its polyphenol and flavonoid content, which plays a crucial role in wound healing activity. Traditionally, extracts of various parts of *T. populnea* plant have been used to treat various skin diseases including wound healing. Therefore, we made a topical gel containing alcoholic extracts of the leaves of *T. populnea* for antimicrobial and wound healing activities. **Materials and Methods:** Extracts of *T. populnea* were successfully obtained by Soxhlet extraction. Phytochemical screenings were performed to estimate the presence of different metabolites. Combinations of carbopol and propylene glycol were optimized through $3^2$ factorial design for desirable gel characteristics. **Results and Discussions:** Formulated gel was pale brown in color having viscosity of 90,300 cps with acceptable spreadability and extrudability index. Antimicrobial studies showed inhibition activity. The *in vivo* wound healing studies demonstrated comparable healing properties with respect to marketed Soframycin gel. Furthermore, histopathological studies on Wistar rats also confirmed the same. **Conclusion:** It can be concluded that the formulated gel is beneficial in topical applications, which can be a beacon for new skin regeneration and wound healing therapies that focuses on herbal remediation.

**Key words:** Antimicrobial, histopathology, gel, *Thespesia populnea*, wound healing

## SUMMARY

- Wound care has always been an active area of research where several miraculous healing potentials of traditional plants have still been scientifically unanswered. *T. populnea* is a species of flowering plant belonging to the mallow family, Malvaceae, which is one of the traditionally used medicines. Several metabolites were found to be present in the alcoholic extract of *T. populnea*, i.e., saponins, tannins, and phenols. The prepared gel containing *T. populnea* extract showed antibacterial activity against both *E. coli* and *S. aureus*. Finally, the *in vivo* wound healing study on the incision wound model revealed that the gel containing *T. populnea* extract healed the wound faster and healing was observed at the end of the 16th day.




**Abbreviations used:** MHA: Mueller Hinton Agar; FDA: Food and Drug Administration; WHO: World Health Organization

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## INTRODUCTION

Wound care is constantly evolving with the advances in medicine. Wound management has several challenges like the emergence of resistant pathogens and hence search for the ideal wound healer still continues.[1] Several reports on the use of herbal and traditional medicine in wound care management have been documented.[1-3] Plants have anecdoctal claims for wound healing. But their efficacy and mechanism of action are not studied. Thus, there is a need of revisiting traditional medicines and provide new rigorous scientific evidences to justify their potential use. The medicinal value of *Thespesia populnea (T. populnea)* is mentioned in traditional medicine.[4] Different portions of *T. populnea* are used to treat ulcers, psoriasis, scabies, and wounds among other skin conditions.[3-6] *T. populnea* fruit aqueous extract has been shown to have significant wound healing activity.[7] Glycosides, flavonoids, alkaloids, phytosterols, quercetin, rutin, and lupeal have been found in the leaves of *T. populnea*.[8,9]

Therefore, in the present study, the possible effect of gel containing *T. populnea* alcoholic extracts on the healing of incision wounds in Wistar rats has been examined. The alcoholic extracts of the leaves of *T. populnea* have been separated by a Soxhlet extractor. Screening



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of metabolites has been performed through physico-chemical characterization. Furthermore, Carbopol gel containing the chosen extract of *T. populnea* has been optimized and formulated. The gels showed potential wound healing activity when compared with marketed Soframycin gel. Also, histopathology suggested reduction of inflammatory cells, which indicates the faster healing potential of the formulated gel containing *T. populnea* extract.

## MATERIALS AND METHODS

### Materials

Carbopol 934, propylene glycol, triethanolamine, ethanol, and methanol were purchased from Cayman Chemicals, India. Methylparaben and propylparaben were obtained from Alta Laboratories Ltd., Mumbai. Sodium hydroxide was purchased from AG Traders, Pune. Anesthetic ether was purchased from Sherad Laboratories, Hyderabad. The ethics committee is obtained VSCP/EC/022/2022/1-2(Date of Approval 01.02.2021).

### Preparation of extract

Fresh *T. populnea* leaves were harvested and dried for 1 week in September 2019 in Harapanahalli, Karnataka, India. Table 1 details the properties of the leaves. The dried leaves (250g) were pulverized and sieved 22 times. In a Soxhlet extractor, the powder was extracted sequentially with hydroalcoholic solvent (Ethanol, 70% v/v) and distilled water. The extracts were vacuum-dried, and the dried mass's weight was reported. Table 2 shows the total ash content of *T. populnea* extracts.

### Phytochemical screening

A full evaluation of both primary and secondary metabolites derived as a result of plant metabolism should be included in a systematic and comprehensive study of crude medicines. Thus, phytochemical extraction has been performed for separating active plant materials or secondary metabolites using solvent extraction procedure. Different qualitative phytochemical screening tests[10] were performed for confirming the presence of the metabolites in *T. populnea* leaf extract.

**Table 1:** Characterization of the *Thespesia populnea* leaf powder

| Leaf powder | Characteristics |
|---|---|
| Color | Dark green |
| Odor | Characteristic |
| Taste | Slightly bitter |
| Loss on Drying | 4.12% |

**Table 2:** Determination of ash content of the powdered leaf of *Thespesia populnea*

| Ash determination | Ash values (% w/w) |
|---|---|
| Total ash | 5.5 |
| Acid insoluble ash | 0.75 |

### Quantification of total phenolic and flavonoid content

Total phenolic content was determined using the Folin–Ciocalteau reagent.[11] The concentration of phenolic content was measured using gallic acid total equivalents unit's mg/g. (GAE) with respect to standard gallic acid calibration curve. Total flavonoid contents in the extracts were determined using quercetin by aluminium chloride colorimetric assay. The total flavonoids content was expressed as quercetin equivalents.

### Preformulation studies of gel preparation

Optimization of carbopol gel and propylene glycol compositions was estimated using factorial design. A $3^2$ randomized full factorial design was performed where the amount of carbopol (X1) and propylene glycol (X2) were taken as independent variables while viscosity (Y1), spreadability (Y2), and extrudability (Y3) were taken as dependent variables. The factors were studied at three levels [Table 3]. Design Expert 8 and Statgraphics Centurion 16 softwares were used.

### Preparation of topical gel of *T. populnea* extract

Different combinations of carbopol 934 and propylene glycol were screened during the factorial design for desirable gel consistency. Optimized composition has been opted for gel preparation where carbopol 934 was the gelling polymer while propylene glycol was acted as a humectant during the gel formulation [Table 4]. Furthermore, 4% of *T. populnea* alcoholic extract was loaded into the formulated topical gel. The optimized composition of the gel ingredients has been provided in Table 5.

### Characterization of gel of *T. populnea* extract

Physical appearances of the formulated gel were evaluated through visual perception and also with the simple microscope. A digital pH meter is used to find out the pH of gel formulations. The pH of each formulation was investigated in triplicate and the average reading was recorded. Viscosity of gel was measured by use of Brookfield viscometer (LVDV-II + Pro). The sufficient quantity of formulated gel was filled in sample holder separately. The height of the gel that was filled in the sample holder should sufficiently allow to dip the spindle. Viscosities of the gels were recorded by adjusting the rotating speed of the spindle at 2.5 rpm. All the formulation of gels were stored in container and visually observed to identify for their appearance of any type of aggregates in the gel formulations.

### Estimation of antimicrobial activity of formulated gel

Antibacterial activity was measured using agar disk diffusion method.[12] The solid media used was Mueller Hinton Agar (MHA) and the solution was heated on a hot plate with continuous stirring. Optimum layer of agar solution was poured into petri dish and allowed to solidify. Three wells had been punctured in both the petri dishes containing *S. aureus* and *E. coli* colonies. Furthermore, formulated gel and marketed Soframycin gel were placed into different wells of the petri dishes. The diameter of

**Table 3:** Variables and their levels used in the $3^2$ factorial design for optimization of Carbopol 934 (Gelling Polymer) and Propylene Glycol (Humectant)

| Level (code value) | Actual value | | Responses | | |
|---|---|---|---|---|---|
| | X1 carbopol (g) | X2 PG (mL) | Y1 | Y2 | Y3 |
| −1 | 0.5 | 5 | Viscosity | Spreadability | Extrudability |
| 0 | 1 | 10 | | | |
| +1 | 1.5 | 15 | | | |

the inhibitory zone formed was measured using a caliper to determine the effectiveness of the formulations. The experiments were performed in triplicats for both bacterial strains.

## Wound healing studies

Animals were divided into three groups of six rats each. Animals were kept in separate cages and a standard wound of a uniform 2-cm diameter was formed on the dorsal area of the rats. Group A rats were not treated and served as control. Group B was treated with marketed Soframycin, and Group C with formulated *T. populnea* extract gel, once a day topically. Until day 16, the % wound closure, epithelization time, and scar area on complete epithelization were measured at predetermined intervals.

## Histopathology studies

Wound tissue collected was subjected to detailed histopathological processing. Tissue was fixed in formalin and processed. These tissue blocks were cut at 4–5 μ in thickness and stained with hematoxylin and eosin and finally mounted with digital picture exchange. Slides were then studied under a light microscope and photographs were taken.

# RESULTS AND DISCUSSION

## Preparation and phytochemical evaluation of *T. populnea* leaf extracts

Qualitative phytochemical screening of all extracts of *T. populnea* leaves revealed the presence of several secondary metabolites. The phenolic content (%) of *T. populnea* leaf extracts was found to be 47.23 ± 1.32 with respect to gallic acid equivalent. Total flavonoid content of *T. populnea* leaf alcoholic extracts was found to be 60.59 ± 1.50 with respect to quercetin. Presence of these constituents in the extracts indicates its potential antioxidant and anti-inflammatory characteristics, which could accelerate the process of wound healing.[13]

**Table 4:** Results of the responses (viscosity, spreadability, and extrudability) as obtained during evaluation of the 13 factorial batches

| Viscosity Y1 (cps) | Spreadability Y2 (g.cm/s) | Extrudability Y3 (%) |
|---|---|---|
| 93,400 | 11.76 | 79 |
| 90,200 | 10.40 | 72 |
| 90,100 | 10.82 | 74 |
| 98,800 | 8.9 | 64 |
| 85,200 | 12 | 83 |
| 94,300 | 9.8 | 69 |
| 88,000 | 11.36 | 87 |
| 86,300 | 9.37 | 66 |
| 90,300 | 10.56 | 73 |
| 90,200 | 10.80 | 74 |
| 80,400 | 13.39 | 89 |
| 90,100 | 10.50 | 73 |
| 84,500 | 12.53 | 88 |

**Table 5:** Composition of the optimized gel containing alcoholic extract of leaves of *T. populnea*

| Ingredients | Qty (% w/w) | Category |
|---|---|---|
| Alcoholic extract of leaves of *Thespesia populnea* | 4 | Antimicrobial, wound healing. |
| Carbopol 934 | 1.0 | Gelling polymer |
| Triethanolamine | qs | pH neutralizer |
| Propylene glycol | 10.0 | Humectant |
| Methyl parabens | 0.10 | Preservative |
| Propyl parabens | 0.02 | Preservative |
| Water | Qs 100 | Medium |

## Preparation and characterization of topical gel of *T. populnea* extract

The gel formulation containing *T. populnea* extract was prepared successfully. Color of the gel was pale brown with semisolid appearance. Also, the gel was smooth and homogeneous with characteristic odor. pH of the gel formulation containing *T. populnea* extract was found to be 6.78 ± 0.01. Role of pH is a crucial factor in the process of wound healing, higher pH (more than 8.4) is favorable for bacterial infection and hence can worsen the wound health. Efforts to make the local wound pH in the range of 6–8 are better for re-epithelization and hence faster wound healing.[14] Viscosity (cps) was 90,300 ± 200 cps with spreadability (g.cm/s) of 10.56 ± 1 and extrudability (%) of 73 ± 2, respectively. Optimum spreadability and extrudability are crucial factor in the successful gel formulation. Also, good extrudability and spreadability determine the compliance and successful delivery of active ingredients to the affected site. Thus, it can be estimated that the prepared gel has optimum spreadability and extrudability, which enables successful dispatch of *T. populnea* extract from the formulation and hence useful in wound healing application.

## Antimicrobial study of the formulated gel

The antibacterial activity test of *T. populnea* extract was carried out on *E. coli* bacteria.[15] and *S. aureus*.[16] The media used in the test was Mueller Hinton Agar (MHA) recommended by both Food and Drug Administration (FDA) and World Health Organization (WHO).[17] The results of zone of inhibition showed antibacterial activity of the prepared gel against both *S. aureus* (a gram positive) and *E. coli* (a gram negative) [Figure 1]. *T. populnea* extract contains phenol, which plays an important role in antibacterial activity against both gram-negative and gra-m positive bacteria. The hydroxyl group (–OH) inhibit the metabolism of bacteria, interact with cell components, and reduce growth.[17,18] Therefore, it can be concluded that the prepared gel has antimicrobial properties which is crucial for rapid wound healing and proper wound care management.

## Wound healing studies

Figure 2 shows wound healing pattern upon application of marketed Soframycin, formulated gel and control (no treatment) in the duration between days 1 to 16. In terms of overall external appearance, the wound closure appeared to be quite evident for treated formulations (i.e., marketed Soframycin and formulated gel containing *T. populnea* extract) as compared with no treatment (control group). Figure 2 clearly shows that for marketed Soframycin and formulated gel containing *T. populnea* extract, the healing was quick and the wounds were almost healed after 16th day of application. Contraction of the wounds (%) was monitored

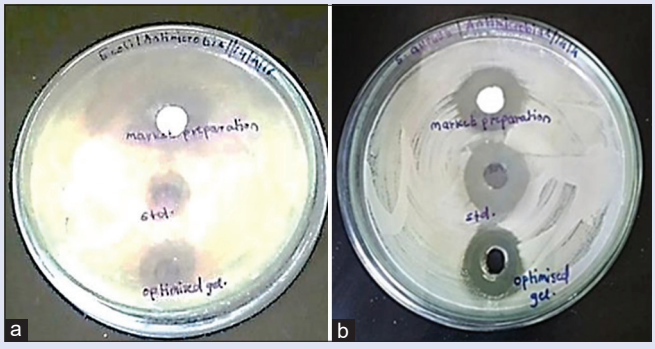


**Figure 1:** Zone of inhibition against *E. coli* (a) and *S. aureus* (b) by Standard (Streptomycin), Marketed Soframycin and formulated gel containing alcoholic extract of *T. populnea* leaves

for the duration of 16 days ($n$ = 6). Growth of the epidermal layer from margin to center was faster, which resulted in the contraction of wound area. *T. populnea* extracts contain the constituents (such as saponins, tannins, and flavanoids) which possess antioxidant and anti-inflammatory activity. It has been reported that saponin not only promotes re-epithelialization of the wound but also effectively inhibits inflammatory reactions during the early phase, and promotes matrix synthesis throughout the wound healing process.[19] Tannins possess antimicrobial and anti-inflammatory activity, which could aid in the enhancement of wound healing properties.[20] Similarly, flavonoids have beneficial anti-inflammatory effects and they also protect cells from oxidative damage.[21] It has been reported that the quercetin present in the leaves of *T. populnea* with its anti-inflammatory properties might have helped the wound to heal with minimum scarring.[4] Also, phenolic compounds were found to accelerate wound healing by regulating anti-inflammatory and oxidative markers.[22] Faster wound healing from formulated gel containing *T. populnea* extract can be attributed to the synergistic effects of the phytochemical ingredients present in the extract, which are proven to be effective in enhanced wound healing [Table 6].

## Histopathology studies

Figure 3 shows the histopathological images for H and E staining (a) normal architecture of the skin, (b) wounds of control group, wounds treated with (c) gel containing *T. populnea* extract, (d) marketed Soframycin. Figure 3a dermis, epidermis, hair follicles, and sebaceous glands (as indicated by red arrow). Figure 3b indicates infiltration of inflammatory cells as shown by contrast spots (as indicated by red arrow). However, in case of gel containing *T. populnea* extracts, the reduction in inflammatory changes were observed (as indicated by red arrow) [Figure 3c]. Also, for marketed Soframycin, the reduction of inflammatory cells was observed and onset of proliferation was noticed with new connective and granulation tissue being formed (as indicated by red arrow). Furthermore, newly formed epithelial layer was also visible [Figure 3d]. Transition from the inflammatory to the proliferative phase is important in wound healing process.[23] The inflammatory phase leads to hemostasis and onset of innate immune system, which defends us against invading pathogens.[24] Extended inflammation is harmful as it causes excessive scarring.[25] Thus, reduced inflammation phase and early onset of proliferation arerequisite for proper and faster wound care management. The results obtained from the histopathology indicate that the gel formulation containing *T. populnea* extract enables reduction in inflammatory changes. Thus, early onset of proliferation is feasible and hence rapid wound healing. This is also in agreement with the wound healing studies [Figure 2] where the healing for wounds treated with the formulated gel containing *T. populnea* extract and marketed Soframycin was comparable.

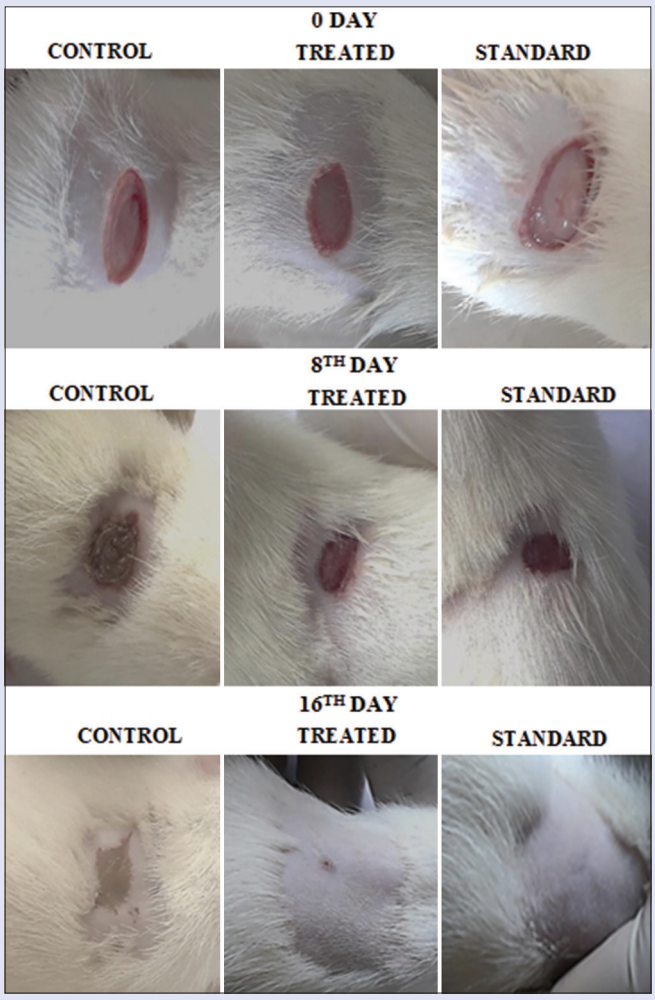


**Figure 2:** Macroscopic view of the dorsum of rats with incision induced wounds and their healing over the duration of 16 days

## CONCLUSION

Wound healing is a complex physiological process that occurs to restore skin integrity after injury. Wound care has always been an active area of research where several miraculous healing potentials of traditional plants has still been scientifically unanswered. Thus there is a need of revisiting traditionally used medicine and systematically review their scientific

**Table 6:** Wound healing studies of formulated gel containing alcoholic extract of *T. populnea* leaves in comparison with marketed Soframycin gel.

| Days | % Contraction ($n$=6) | | |
|---|---|---|---|
| 0 | Control | Standard (Soframycin) | Treated *T. populnea* gel |
| 4 | 16.2±3 | 18.6±2 | 16.9±3 |
| 8 | 20.54±0.5 | 45.9±1 | 40.5±0.6 |
| 12 | 40.3±1 | 92.4±0.5 | 87.3±1 |
| 16 | 60.2±0.5 | 100 | 98±1 |

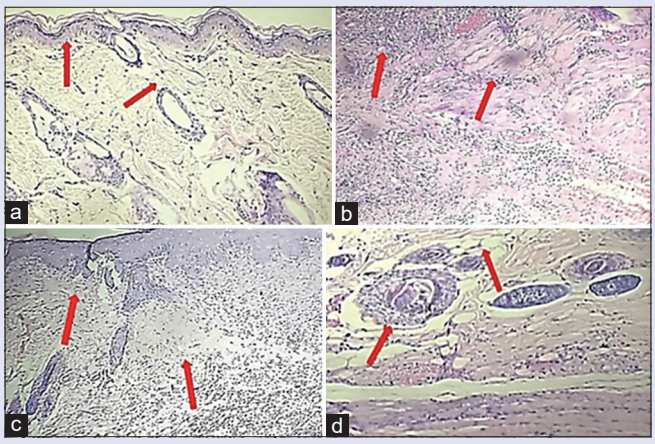


**Figure 3:** Histopathology images of the tissues obtained by incision induced wounds from the Wistar rats (a) Normal architecture of the skin, (b) Control (non-treated group), (c) Treated with formulated gel containing *T. populnea* extract, (d) Treated with marketed Soframycin

evidences. In this paper, we have focussed on gel preparation of *T. populnea* extract. *T. populnea* is a species of flowering plant belonging to the mallow family, Malvaceae, which is one of the traditionally used medicines. *T. populnea* extract was successfully isolated and characterized. Several metabolites were found to present in the alcoholic extract of *T. populnea*, i.e., saponins, tannins, and phenols. Furthermore, compositions of carbopol and propylene glycol were optimized via $3^2$ factorial designs. Then, the carbopol (gelling agent) and propylene glycol (humectant) gel containing *T. populnea* extract was successfully prepared. The prepared gel containing *T. populnea* extract showed antibacterial activity against both *E. coli* and *S. aureus*. Finally, the *in vivo* wound healing study on the incision wound model revealed that the gel containing *T. populnea* extract healed the wound faster and healing was observed at the end of $16^{th}$ day. Histopathology showed a reduction in inflammatory cells for both the wounds treated with a gel containing *T. populnea* extract and marketed Soframycin, respectively. The study confirms the ability of gel containing *T. populnea* extract to promote wound healing both qualitatively and quantitatively. It can be concluded that gel containing *T. populnea* extract is beneficial for topical wound management.

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## Conflicts of interest

There are no conflicts of interest.

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