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Vanishree Subhanreddy
M.Pharm, Department of Pharmacology, H.K.E's Matoshree Taradevi Rampure Institute of Pharmaceutical Sciences, Kalaburagi, Karnataka, India

Dr. Arati Malpani
Department of Pharmacology, H.K.E.'S Matoshree Taradevi Rampure Institute of Pharmaceutical Sciences, Kalaburagi, Karnataka, India

Nisar Ali
Department of Pharmacology, H.K.E.'S Matoshree Taradevi Rampure Institute of Pharmaceutical Sciences, Kalaburagi, Karnataka, India

Corresponding Author:
Vanishree Subhanreddy
M.Pharm, Department of Pharmacology, H.K.E's Matoshree Taradevi Rampure Institute of Pharmaceutical Sciences, Kalaburagi, Karnataka, India

Wound healing activity of ethanolic extract of pods (fruit) of *Acacia concinna* Linn. as a traditional medicine in Wister albino rats

Vanishree Subhanreddy, Dr. Arati Malpani and Nisar Ali

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Abstract
In Ayurveda pods of *Acacia concinna* L. were claimed to possess wound healing potential. The present study was undertaken to evaluate the ethanoic extract of pods of *Acacia concinna* in the form of an ointment with two different concentrations (5% and 10% w/w ointment of pods extract in simple ointment base) were evaluated for wound healing activity in rats using excision and incision wound model. Both concentrations of the ethanolic extract ointment showed significant responses in both the wound types (excision and incision) when compared with the control group. 5% & 10% ointment of EEAC were applied topically on the wounds of rats and observed wound contraction, percentage of wound closure & period of epithelization, in excision model and tensile strength in incision model. Both 5% & 10% (w/w) extract showed significant wound healing activity by increasing percentage of wound contraction, reducing epithelization period and tensile strength. The wound healing effect was dose dependent. The wound healing activity of EEAC may be due to the phytoconstituents present in extract such as flavonoids, saponins, alkaloids & tannins.

Keywords: *Acacia concinna* L., wound healing, excision wound, incision wound, soframycin

Introduction
Wound is one of the most common disease often having severe complications in relation to health and posing high costs for therapy. To establish the integrity of the damaged tissue, the series of events must be progressed orderly in a well-controlled manner, otherwise may be causing physical disability even leading to death [1].
Wound healing is the process of repair that follows injury to the skin and other soft tissues. Following injury, an inflammatory response occurs and the cells below the dermis (the deepest skin layer) begin to increase collagen (connective tissue) production. Later, the epithelial tissue (the outer skin) is regenerated. There are three stages in process of wound healing: those are inflammation, proliferation, and remodeling [2].
The proliferation phase is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelialization, and wound contraction. Angiogenesis involves new blood vessel growth from endothelial cells. In fibroplasia and granulation tissue formation, fibroblasts excrete collagen and fibronectin to form a new, provisional extracellular matrix. Subsequently, epithelial cells crawl across the wound bed to cover it and the wound is contracted by myofibroblasts, which grip the wound edges and undergo contraction using a mechanism similar to that in smooth muscle cells [3].
Currently available methods of wound management including irrigation, debridement, antibiotics, proteolytic enzymes and tissue grafts are found to be associated with major drawbacks such as invasiveness and are expensive. Emergence of resistance strains along with lack, high cost and retarded rate of newly generated antibiotics increase wound related mortality and morbidity [4]. Plants or chemical entities derived from the plants are cheaper with minimum side effect need to be identified and formulated for treatment and management of wounds. In this direction, a number of herbal products are being investigated by many researchers. Various herbal products have been used in management and treatment of wound over the years [5].
Acacia concinna Linn. (Leguminosae) is a medicinal plant that grows in topical rainforests of southern Asia and the fruits of this plant are used for washing hair, for promoting hair growth, expectorant, emetic, and purgative. Although the pods of this plant known to contain several saponins based on acacic acid, previous chemical examination resulted in the identification of flavonoids 1 and monoterpenoids [6].

There are versatile uses of this plant (leaf, stem and pods) in different aspects of life. The leaves and pods are used to treat cuts, wounds, oral diseases and antidandruff. Leaves of shikakai act as a purgative, liver stimulant and improves taste. The decoction of shikakai pods used to treat constipation, abdominal pain, indigestion and flatulence and infusion of the leaves of the plant has also been used for therapy of jaundice in the traditional Indian medicines. The pulp of the fruit (pods), without the seeds, is used as a diuretic and emetic, while the seeds are reputed to make delivery [7, 8].

Ayurveda reports utility of *Acacia concinna* in enhancing wound healing process, however, the literature survey revealed that no systemic study had been carried out on wound healing activity. Hence, in present study, an effort was made to establish wound healing potential of the plant using different models.

Materials and Methods

Preparation of plant extract

The fruit pods of *Acacia concinna* L. will be collected, shade dried. The dried pods will be pulverized separately into coarse powder by a mechanical grinder. The resulting *Acacia concinna* pods powder (100 g) was defatted with 150 ml of petroleum ether and extracted with ethanol by hot percolation method using a Soxhlet apparatus at 40 °C to obtain the ethanolic extract of the plant. The filtrate of the extract was concentrated and dried at a temperature of 30 °C. The percentage yield was calculated and reported.

Animals

Wister albino rats (150-200 g) of either sex were obtained from Mahaveer Enterprises, Hyderabad, Telangana, India (1656/PO/Bt/S/12/CPCSEA). They were maintained in an HKES MTRIPS animal house at a temperature of 25 ± 1° C and a relative humidity of 45% to 55% under a 12-hours light and 12-hour dark cycle. The animals had a free access to food pellets, and water was available *ad libitum*. Prior permission from the IAEC was obtained for the conduct of the experiments (IAEC approval no. HKES/MTRIPS/IAEC/122/2021-22).

Acute dermal toxicity test

The acute dermal toxicity testing of the ethanolic extract of pods of *Acacia concinna* was done by applying the ointment on shaved back in rats. The OECD guidelines no.402 was fallowed for the study [9].

Preliminary Phytochemical Screening

An ethanolic extract of the pods of *Acacia concinna* was qualitatively tested for the detection of alkaloids, flavonoids, saponins, tannins and phenolic compounds [10].

Wound Healing Activity

Formulation of simple ointment base and extract ointment Procedure

Hard paraffin was placed into an evaporating dish and melted on a water bath. The dish was removed and the other ingredients (Cetosteryl alcohol, wool fat, white soft paraffin) were added in descending order of melting point until all were melted. The mixture was continuously stirred to ensure homogeneity, but at the same time gently to avoid incorporation of excess air. Lastly added the accurately weighed ethanolic extract of pods of *Acacia concinna* L. to the simple ointment base.

Table 1: Preparation of simple ointment base

Sr. No.	Ingredients	Quantity
1.	Hard Paraffin	5 g
2.	Cetosteryl Alcohol	5 g
3.	Wool Fat	5 g
4.	White Soft Paraffin	85 g
	Total	100 g

Wound healing models

In the present study, for evaluation of wound healing activity of ethanolic extract of pods of *Acacia concinna* Linn. excision and incision wound model were used. The animals were devided into four major groups those are control, standard, Test-1 (EEAC-5%), Test-2 (EEAC-10%) with six animals in each group. The control group was treated with simple ointment base. The standard group was treated with soframycin ointment (1% w/w soframycin). The test group treated with ointment in different concentrations of extract *viz*. 5% (w/w), and 10% (w/w) incorporated in simple ointment base, in excision and incision wound models [11].

Excision wound model

Animals have been anesthetized with ketamine injection (50 mg/kg ip) and back hair of the animals have been shaved. An excision wound was inflicted by cutting away 500 mm^2 full thickness of a pre-determined area on depilated back of the rat. Epithelialization period was noted as the number of days after wounding required for the scar to full off leaving no raw wound behind. Wound contraction rate was monitored by planimetric measurement of wound area on alternate days. This was achieved by tracing the wound on a graph paper. Reduction in the wound area was expressed as percentage of the original wound size. The degree of wound healing was calculated as percentage closure of the wound area from the original wound area using fallowing formula [12].

$$\text{Percentage of wound closure (\%)} = \frac{\text{wound area on day 0} - \text{wound area on day n}}{\text{wound area on day 0}} \times 100$$

Where n - number of days [4^{th} day, 8^{th} day, and 12^{th} day]. The mean percentage of wound closure and standard error were calculated in control and various treated groups [12].

Fig 1: A circular excision wound on the day 0

Incision wound model

Anesthesia was given by using ketamine (50 mg/kg) and animals were secured to operation table in its natural position. A paravertebral straight incision of 4 cm was made through the entire thickness of the skin of the vertebral column with the help of sharp scalpel blades. Care was taken to see that the incisions were done properly to the vertebral column. After complete homeostasis, the wounds were closed by means of interrupted sutures placed at equidistant points about 1 cm apart, using 4-zero silk thread and straight round needle. Wounds were then mopped with cotton swabs in saline and were caged individually and that day was considered as day 0. From day 1, the ointments were applied once a day for 9 days. Removal of sutures was done on 8th post wounding day. Breaking tensile strength was measured on 10th post wounding day by continuous, constant water flow technique.

Fig 2: Incision wound on day the 0

Statistical analysis

Data was expressed as mean ± SEM & statistical analysis was carried out by one-way ANOVA followed by Dunnett's test using Graph Pad Prism version 09. P value<0.05 was considered as significant [13].

Table 2: Mean raw wound area (mm^2) of excision wounds at different time intervals of various groups

Days	Mean raw wound area (mm^2)			
	Control	Standard (soframycin)	Test-1 (EEAC 5%)	Test-2 (EEAC 10%)
0	498.0±0.85	497.3±0.88	497.7±0.84	498.0±0.93
4	409.2±14.72	333.8±8.66****	366.8±6.651*	345.6±5.388**
8	181.2±9.06	115.0±6.71****	134.8±6.41**	127.8±9.28***
12	101.1±10.15	44.88±4.43***	67.82±6.13**	54.90±4.69***

n=6; Values are expressed as mean ± S.E.M; *p<0.05, **p<0.01, ***p<0.001 compared to control group

Table 3: Percentage closure (%C) of excision wounds at different time intervals of various groups

Days	Percentage closure (%C)			
	Control	Standard (soframycin)	Test-1 (EEAC 5%)	Test-2 (EEAC 10%)
0	----	----	----	----
4	17.83±2.879	32.83±1.801****	26.28±1.294*	30.55±1.142***
8	63.53±1.803	76.83±1.348****	72.88±1.318**	74.20±1.783***
12	79.45±1.971	90.92±0.894****	86.30±1.237**	88.82±1.074***

n=6; Values are expressed as mean ± S.E.M; *p<0.05, **p<0.01, ***p<0.001 compared to control group

Table 4: Time (Days) for complete wound closure (Epithelialization) of Excision wound

Animal number	Epithelialization Days			
	Control (simple ointment)	Standard (Soframycin)	Test-1 (EEAC 5%)	Test-2 (EEAC 10%)
1	15	7	12	9
2	13	11	10	12
3	16	8	12	10
4	15	10	10	12
5	13	9	11	10
6	14	8	13	9
Mean ± SEM	14.33±0.4944	8.833±0.6009****	11.33±0.4944**	10.33±0.5578***

n=6; Values are expressed as mean ± S.E.M; *p<0.05, **p<0.01, ***p<0.001 compared to control group

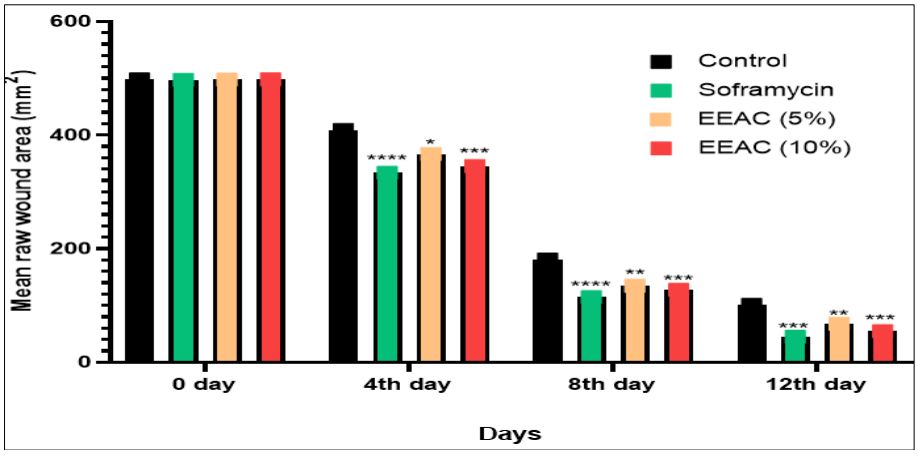

Fig 3: Mean raw wound area (mm^2) of excision wounds at different time intervals of various groups

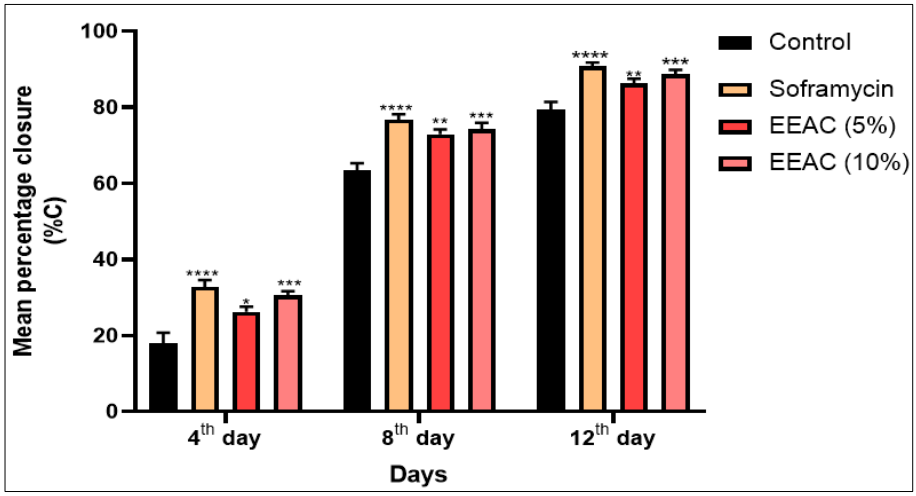

Fig 4: Percentage closure (%C) of excision wounds at different time intervals of various groups

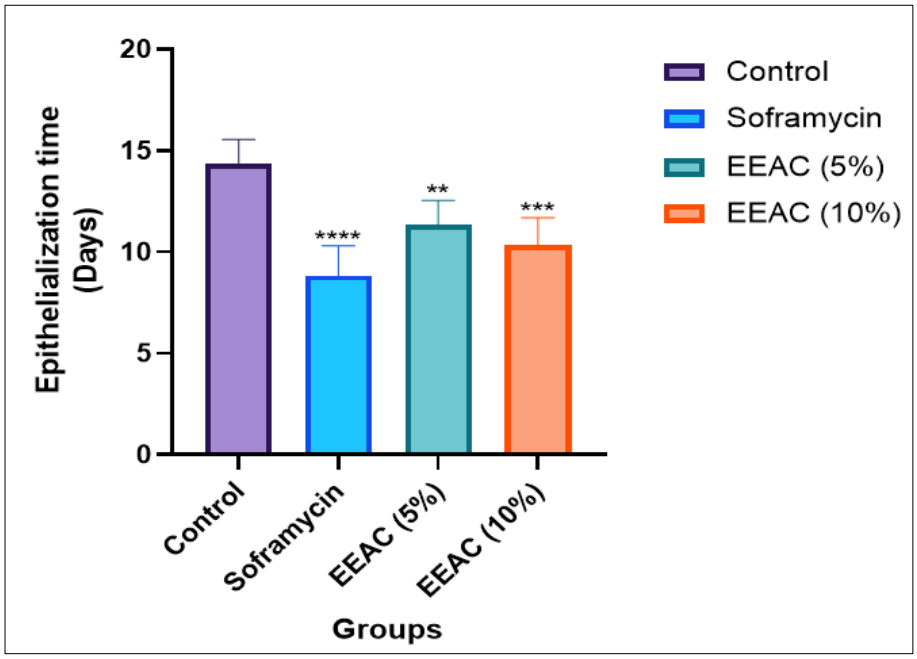

Fig 5: Effect of EEAC on Epithelialization (Excision Model)

Table 5: Tensile strength (g) after 10th post wounding day of resutured incision wound in different groups

Animal number	Tensile strength (g)			
	Control (simple ointment)	Standard (soframycin)	Test-1 (EEAC 5%)	Test-2 (EEAC 10%)
1	280	310	300	320
2	300	420	357	400
3	360	461	350	431
4	310	471	330	441
5	300	450	345	422
6	250	410	330	350
Mean ± SEM	283±9.888	420.3±24.07****	335.3±8.333	394.0±19.84***

n=6; Values are expressed as mean ± S.E.M; *p<0.05, **p<0.01, ***p<0.001 compared to control group

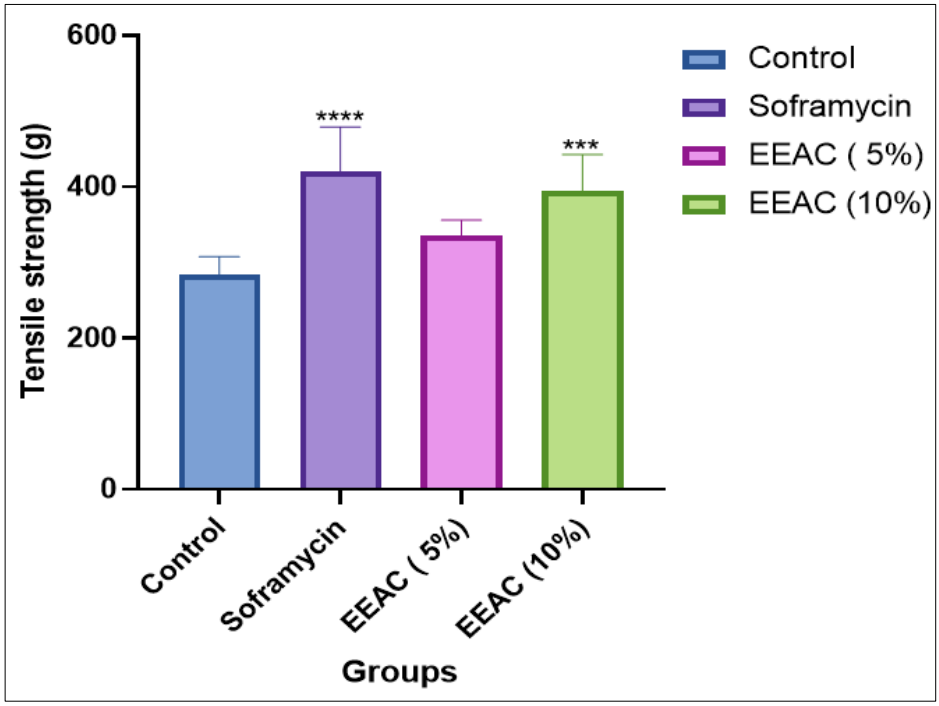

Fig 6: Effect of EEAC on the tensile strength (Incision Model)

Fig 7: Assembly to estimate tensile breaking strength

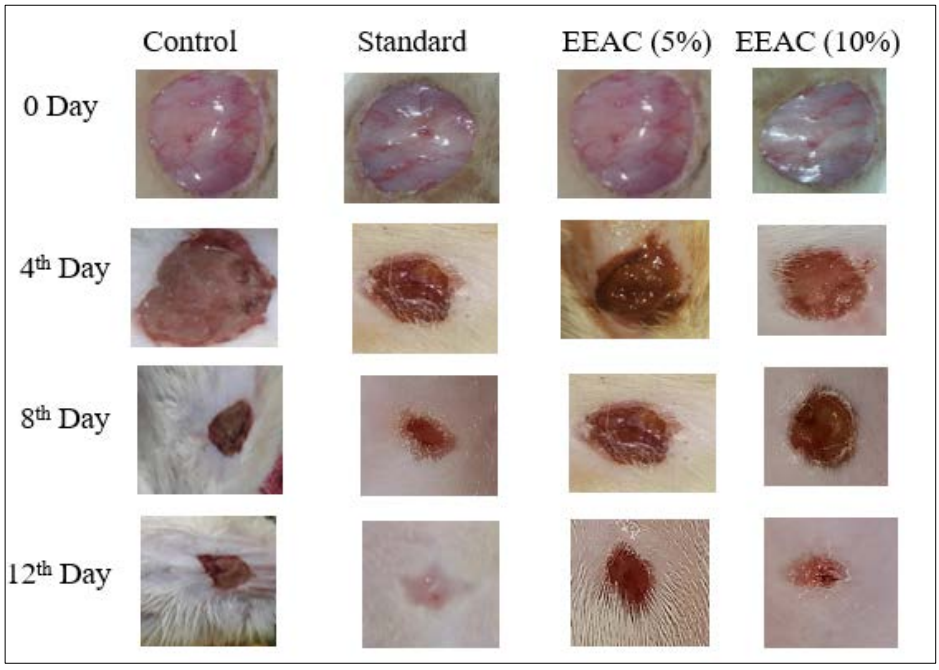

Fig 8: Wound contraction progress in simple ointment, 5% w/w and 10%w/w pods of *Acacia concinna* L. extract and 1% w/w Standard soframycin treated group across post-wounding days in excision model

Results

Phytochemical analysis

The percentage yield to ethanolic extract of pods of *Acacia concinna* was 25%. The preliminary phytochemical assessment of EEAC showed the presence of alkaloids, saponins, flavonoids and tannins, phytosterols, phenolic compounds.

Chemical constituents present in ethanolic extract of pods *of Acacia concinna* L.

Test	Ethanolic Extract
Alkaloids	+
Saponins	+
Tannins	+
Flavonoids	+
Phenolic compounds	+
Terpenoids	-
Phytosterols	+

(+) - Positive, (–) - Negative

Excision wound study

The results of wound healing activity by excision wound model were presented in Table 1 and 2. The values present in the table represents the percentage of wound healing and percentage of wound closure at 4, 8 and 12th day for control (simple ointment treated group), standard (soframycin ointment) and the test groups ethanolic extract of *Acacia concinna* Test-1(EEAC 5%) and Test-2 (EEAC 10%). It is observed that wound contracting ability of animals treated with EEAC 10% was found to be significantly higher as compared to control group. The results epithelization period are also presented in Table 3.

Incision wound study

The effect of wound healing activity in this model was evaluated by determining the tensile strength of the incision wound of different groups. Control treated with simple ointment base, standard group treated with the soframycin ointment and the test group treated with EEAC extracts at different concentrations. The results are presented as mean ± SEM. The animals treated with ointment containing 10% (w/w) of ethanolic extract of *Acacia concinna* significantly high tensile strength as compared to the control group.

Discussion

Wounds are defined as a breach in the continuity of living tissues. Thus, humans cannot escape from an event of injury in their lifetime. Depending upon the causation, site of injury, condition of the patient, extent of trauma etc., the wound could be minor or major. Wound care and maintenance involve a number of measures including dressing and administration of painkillers, use of anti-inflammatory agents, topical and systemic antimicrobial agents and healing promoting drugs [14].

Wound healing and tissue repair are complex processes that involves a dynamic series of events including clotting, inflammation, granulation tissue formation, epithelization, collagen synthesis and tissue remodeling [15].

The preliminary phytochemical assessment of EEAC showed the presence of alkaloids, saponins, triterpenoids, flavonoids and tannins.

Flavonoids helps to reduce the formation of inflammatory metabolites by inhibiting the cyclooxygenase and lipoxygenase activities. Furthermore, flavonoids inhibit neutrophil degranulation, which is a direct way to reduce the release of arachidonic acid by neutrophils and other immune cells, which aids in wound healing. The astringent and anti-microbial properties of these phytochemical might also be responsible for wound contraction and increased epithelization rates. Flavonoids are also responsible for lowering lipid peroxidation by preventing or slowering cell necrosis and improves vascularity, which increases the viability of collagen fibrils by increasing circulation, preventing the cell damage and promoting DNA synthesis [16].

In the present study, the wound healing effect of ethanolic extract of pods of *Acacia concinna* were evaluated in Wistar albino rats. The results of excision wound model indicate that in the first 4 days there is no significant increase in the wound contraction in all the groups as compared to the control group. The results of the 8th and 12th days indicate that there is a significant increase in the percentage of wound contraction in the group treated with EEAC 5%, EEAC 10%, revealing that the extract has ability to induce cellular proliferation.

The increase in tensile strength of wounded skin indicates the promotion of collagen fibers. Highest tensile strength of the wounded skin was observed in the animals treated with EEAC 10% (w/w). The increased tensile strength reveals that disrupted surface are firmly knit by collagen.

The wound healing studies on pods of *Acacia concinna* indicate that the flavonoids play an important role in wound healing process. It can be proposed that, the high content of flavonoids in pods of *Acacia concinna* may be responsible for wound healing activity. The results were concentration dependent.

Saponins also play a vital role in wound healing process. Saponins will promote the re-synthesis of matrix at the site of a skin wound and also effectively inhibit the inflammatory reactions during the early phase, and promotes matrix synthesis throughout the wound healing process. Based upon this information, saponins is beneficial in healing incision skin wounds [17].

Conclusion

This study confirms the significant wound healing activity of ethanolic extract of pods of *Acacia concinna*, which may be due to flavonoids and saponins present in the extract phytoconstituents. This study supports the plant's traditional use as a wound healing activity. However, further research is required to fractionate and isolate the molecule from the extract, and studies are also required to be carried out to know that exact mechanism of the molecule for the wound healing activity.

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