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Research Article

EFFECT OF VARIOUS EXTRACTS FROM *FICUS RETUSA* L. STEM BARK ON WOUND HEALING POTENTIAL

Puneet Bhardwaj[1]*, Nitin Jumnani[2]*, Shalini Singh[3], Ravi Shankar Rao[1]

*[1]Lords International College of Pharmacy, Alwar.

*[2]Alwar Pharmacy College, Alwar.

[3]Gurukula Kangri (Deemed to be University), Haridwar, Uttarakhand, India.

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ABSTRACT

A wound is defined as a disruption in the physiological continuity and structural integrity of a living tissue. It can be caused by physical, chemical, thermal, microbiological, or immunological damage to the tissue. Plants have enormous potential for wound surveillance and care. In many nations, ancient and traditional medicine employs a wide range of herbs to heal wounds. These common agents promote healing and regeneration of the damaged tissue in a variety of ways. The acetone and ethanolic extracts of *Ficus retusa* stem bark have been taken to evaluate the wound healing potential in excision and incision wound models. The parameters studied include the rate of wound contraction, period of complete epithelialization of the excision model, and tensile strength of the incision wound. A one-way ANOVA test was used to analyze the results obtained from the present study and $p<0.05$ was considered significant. Both acetone and ethanolic extracts of *F. retusa* were found to possess significant wound-healing activity, which was evidenced by a decrease in the period of epithelialization, an increase in the rate of wound contraction, and skin-breaking strength. The present study has demonstrated that the acetone and ethanolic extracts of *F. retusa* have properties that render them capable of promoting accelerated wound-healing activity compared with standard drug and normal control.

INTRODUCTION

In clinical practice, wounds, a clinical entity as old as humanity, frequently present challenges. Of course, the quest for knowledge to advance healing never ends. Over the past half-century, a plethora of intriguing research has yielded significant data about healing and the elements that influence it, including medications[1]. An ordered and prompt reparative process that leads to a sustained restoration of anatomic and functional integrity is typically the outcome of acute wounds. Chronic wounds have either not healed in a timely and orderly manner to provide anatomical and functional integrity, or have healed without producing a long-lasting anatomical and functional outcome[2]. The treatment of chronic wounds has a major financial impact on health care and affects the quality of life (QoL) of around 2.5% of the US population[3]. The process of mending after damage to the skin and other soft tissues is known as wound healing[4]. The mechanism of wound healing is intricate and multifaceted, encompassing a series of planned events such as bleeding, clotting, the start of an acute inflammatory response to initial damage, the migration, proliferation, and regeneration of connective tissue and parenchyma cells, the synthesis of extracellular matrix proteins, the remodeling of new parenchyma and connective tissue, and the deposition of collagen[5]. Wound healing can be assessed by physical, mechanical, biochemical, or histological attributes[6] which are mentioned in Fig No. 1. India presents a diverse range of abundant vegetation that is found all around the nation. In traditional medical practices like Ayurveda, Unani,

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and Siddha, herbal remedies have served as the foundation for the treatment and healing of a wide range of diseases and physiological conditions. Both conventional and Western medicine make significant use of plant-based medicinal ingredients. For thousands of years, pharmaceuticals derived from plants have played a role in the development of human healthcare. The management and treatment of wounds can greatly benefit from the use of plants and their extracts[7]. *F. retusa* is an evergreen shrub or tree with rounded branches and a quick growth rate. It can grow up to 15 meters (49 feet) tall with an equal spread. The stunning smooth, light grey trunk, which reaches a diameter of around one meter (3.3 feet), provides strong support for the massively spreading canopy. Traditionally, it has been used as an antihypertensive, hepatoprotective, gastroprotective, antidiabetic, anthelmintic, antimalarial, anti-inflammatory, analgesic, and antibacterial[8]. *F. retusa* leaves, stem barks, and roots are applied topically to cuts and bruises. Salt and dried roots are administered to teeth that are hurting or rotting. Liver disorders can also be treated using roots. Plant materials often have these advantageous effects because of the mixtures of many phytoconstituents. These phytoconstituents are produced and distributed throughout the entire plant or just in particular sections[9]. This research has been conducted by using this well-known medicinal herb *F. retusa* (stem bark) for its wound healing potential using acetone and ethanolic extracts in rat models.

MATERIAL AND METHODS

Plant Material

The stem bark of *F. retusa* (Moraceae) was collected from the local area and the plant material was identified and authenticated by the Botanical Survey of India, Jodhpur. The air-dried plant parts were reduced to a coarse powder and stored in air-tight containers until the time of use.

Preparation of Extract

The stem bark of *F. retusa* was dried under shade, crushed into small pieces, and powdered. The powder was loaded into the Soxhlet extractor and was subjected to successive extraction with petroleum ether, benzene, chloroform, ethanol, and water to get different extracts. The acetone and ethanolic extracts were concentrated to dryness using a Rotary evaporator, giving a yield of 4.10% w/v and 4.42%, respectively, and preserved in a refrigerator. Aliquot portions of the acetone and ethanol extracts of *F. retusa* were weighed and suspended in an appropriate volume of Tween 80 (2% v/v) for use on each day. Fig No. 2 indicates the Soxhlet apparatus for the extraction of different plant extracts.

Experimental Animals

The experiment involved male and female Wistar rats aged 2-3 months and weighing 200-220gm. They were obtained from the animal house of the Lords International College of Pharmacy, Alwar, Rajasthan. The animals were housed in polypropylene cages with steel roofs at standard environmental conditions of 22±3°C, 63±2% relative humidity, and 12h light/dark cycles. They were individually housed and were allowed free access to a standard pellet diet and water *ad libitum*. Their weight was monitored regularly, both before and after the experiment. The rats were anesthetized before and during the infliction of the experimental wounds. The surgical interventions were carried out under sterile conditions using ketamine anesthesia (at a dose of 120mg/kg). The animals were carefully watched for signs of infection. Any rats that showed signs of infection were removed from the study, treated, and replaced with healthy ones.

Acute Toxicity Study of Extracts

The acute toxicity of acetone and ethanolic extracts of *F. retusa* was assessed in Wistar rats weighing between 200 and 220gm. Before the experiment, the animals were fasted overnight using the fixed dose method of OECD guideline no. 420 (Annexure-2d) of the Committee for Control and Supervision of Experiments on Animals (CPCSEA).

Wound-healing Activity

The wound-healing properties of acetone and ethanolic extracts of *F. retusa* stem bark were assessed using excision and incision wound models.

Excision Wound Model

Animals were sedated before and during wound formation. The animals were maintained anesthetized with mild ether throughout the surgical process. After allowing at least 5mm of space between the ears, a 500mm^2 imprint was taken. The skin in the impressed region was meticulously removed to its maximum thickness, resulting in a 500mm^2 wound. The use of a normal saline solution resulted in hemostasis. The wound was treated topically with ointment base, povidone-iodine, and ointment extracts until it healed entirely. All animals were checked regularly for wound fluid, infection, and other anomalies. The raw wound region was traced on a transport paper on the fourth, eighth, twelfth, and sixteenth days, alternately. The criterion for full epithelization was established as the creation of a scar in the absence of a raw wound area. The degree of wound healing was assessed as a proportion of the wound closure area to the initial wound area. The animals were separated into six

groups of five each (males and females). The different groups were treated as follows:

Group I- Control, applied topically (0.5gm), simple ointment.

Group II- Standard, applied topically, 5% w/w Povidone iodine ointment.

Group III- Treated with acetone extract of *F. retusa* 5% w/w ointment topically.

Group IV- Treated with acetone extract of *F. retusa* 10% w/w ointment, topically.

Group V- Treated with ethanolic extract of *F. retusa* 5% w/w ointment, topically.

Group VI- Treated with ethanolic extract of *F. retusa* 10% w/w ointment, topically.

The percentage of wound contraction was determined using the following formula:

Percentage of wound contraction = Initial day wound size-specific day wound size/Initial day wound size.

The number of days required for falling off the scar without any residual of the raw wound gave the period of epithelization.

Incision Wound Model

The Ehrlich and Hunt incision wound model was examined. The animal was fastened to the operating table in its normal position while under mild ether anesthesia. Rats were sedated both before to and throughout the formation of the wound, just like in the model mentioned above. After the dorsal fur of the rats was removed, a longitudinal paravertebral incision wound measuring 6cm was formed. It was then sutured, using surgical thread (no. 000) and a curved needle (no. 11), at intervals of 1 cm, much like in the excision wound model. On both wrapped edges, the thread was continually tightened to guarantee a good closing. The animals were split into groups and given topically applied treatments once daily for nine days, consisting of an extract ointment, a basic ointment, and conventional medications, following the first day of wound formation. The tensile strength was assessed the next day using the continuous water flow method after the sutures were taken out, eight days after the incision. The approach was used to measure the skin-breaking strength on the tenth day.

Group I- Normal control, treated orally with normal saline.

Group II- Standard, (5% w/w) of povidone iodine.

Group III- Acetone extract of *F. retusa* treated orally at 250mg/kg b.wt.

Group IV- Acetone extract of *F. retusa* treated orally at 500mg/kg b. wt

Group V- Ethanolic extract of *F. retusa* treated orally at 250mg/kg b. wt.

Group VI- Ethanolic extract of *F. retusa* treated orally at 500mg/kg b. wt.

Statistical Analysis

The results of the study were subjected to a one-way analysis of variance using the ANOVA test, values with $p<0.05$ were considered significant.

RESULTS

Acute Toxicity Studies

In the acute toxicity studies, no signs of toxicity or mortality were observed at the 2000mg/kg dose level. Both the extracts at 250 & 500mg/kg b.w. dose did not show any toxic side effects or erythema on the skin surface. Thus, the prepared extracts were considered safe for topical administration.

Excision Wound Model

The results of the progress of the wound healing effect induced by *F. retusa* extract ointments (acetone and ethanol at different concentrations; 5% & 10%), and povidone-iodine at a dose of 5% w/w (standard drug) were shown in Table 1. In the excision wound model, 10% acetone and 10% ethanolic extracts exhibited maximum percentage of wound closure (95.4% and 101.5%) as compared with standard drug (Povidone iodine; 102%) and normal control (88%) with significant reduction in the wound area ($p<0.001$), which have shown in Fig No. 3, whereas the percentage of wound closure with 5% acetone and ethanolic extracts were 91.8% and 98.8% respectively on the 16th day as illustrated in Fig No. 4.

Incision Wound Model

Table 2 shows the effects of acetone and ethanolic extracts of *F. retusa* at a dose of 250 & 500mg/kg on wound-healing activity in rats inflicted with incision wounds. In the incision wound model, a significant increase in the wound-breaking strength was observed with the acetone and ethanolic extracts (350.0±5.51, 384.0±4.56 and 418.0±9.37, 489±11.85) at a dose of 250 and 500mg/kg when compared with the normal control (302.7±6.47). The results were also comparable to standard drug i.e., povidone iodine, with a breaking strength of (425.0±5.36). The difference between treated rats with drugs and control was statistically significant ($p<0.05$). It was found that the mean time for epithelization and mean scar area were reduced significantly, there increasing mean tensile strength compared to the control group. The measurement of breaking strength in the incision model is shown in Fig No. 5.

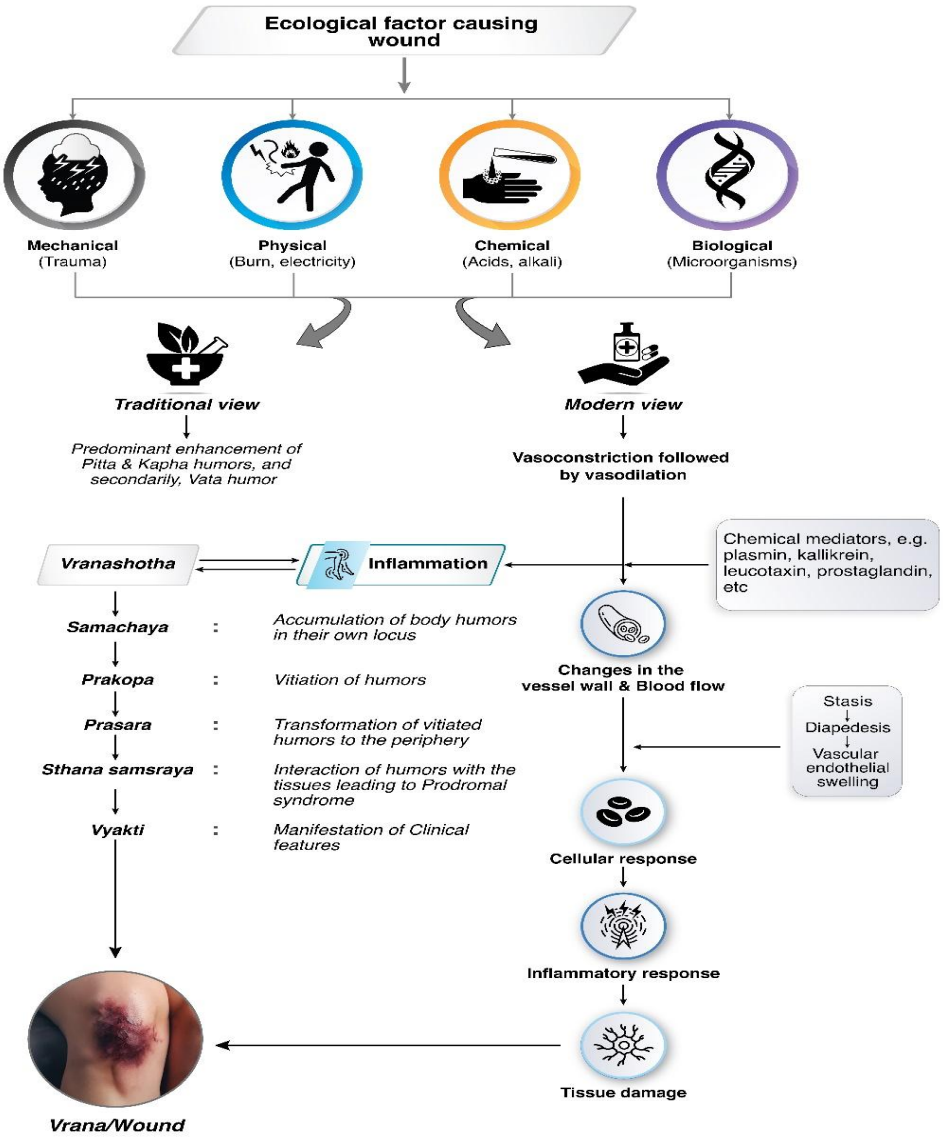

Fig No. 1: Etiology of wound in Ayurveda and modern medicine system

Fig No. 2: Extraction of *Ficus retusa* stem bark through Soxhlet method

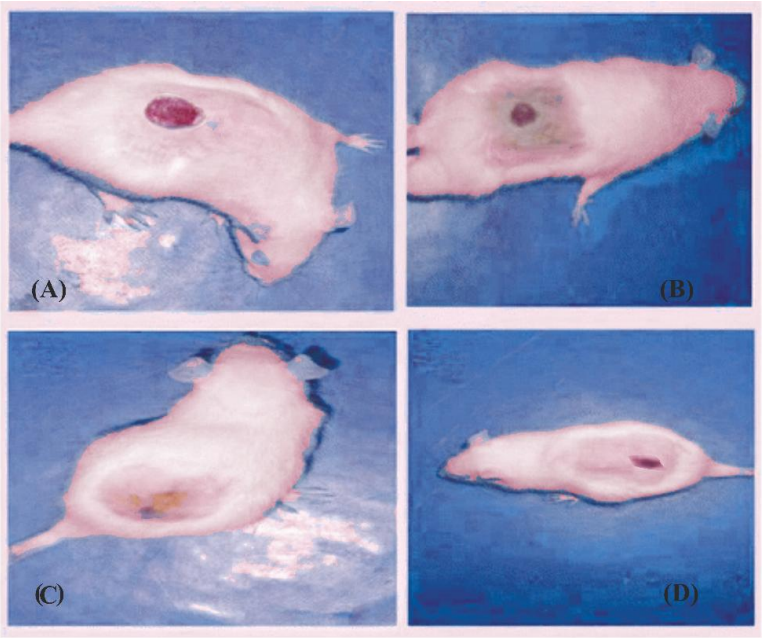

Fig No. 3: Visual illustration of wound healing: (A) Normal Control; (B) Treated with acetone extract 10% w/w from *F. retusa;* (C) Treated with ethanol extract 10% w/w from *F. retusa*; (D) Treated with Povidone iodine ointment (5% w/w) as standard

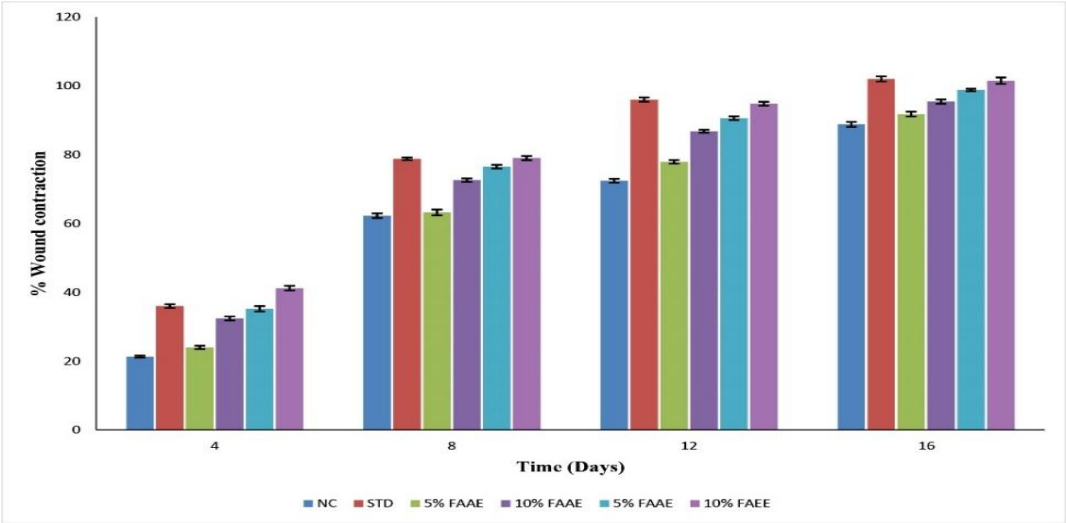

Fig No. 4: Effect of *Ficus retusa* stem bark extracts on wound contractions

*NC= Normal control, STD (Standard drug; 5% w/w) = Povidone iodine, FAAE= *Ficus retusa* acetone extract, FAEE= *Ficus retusa* ethanolic extract

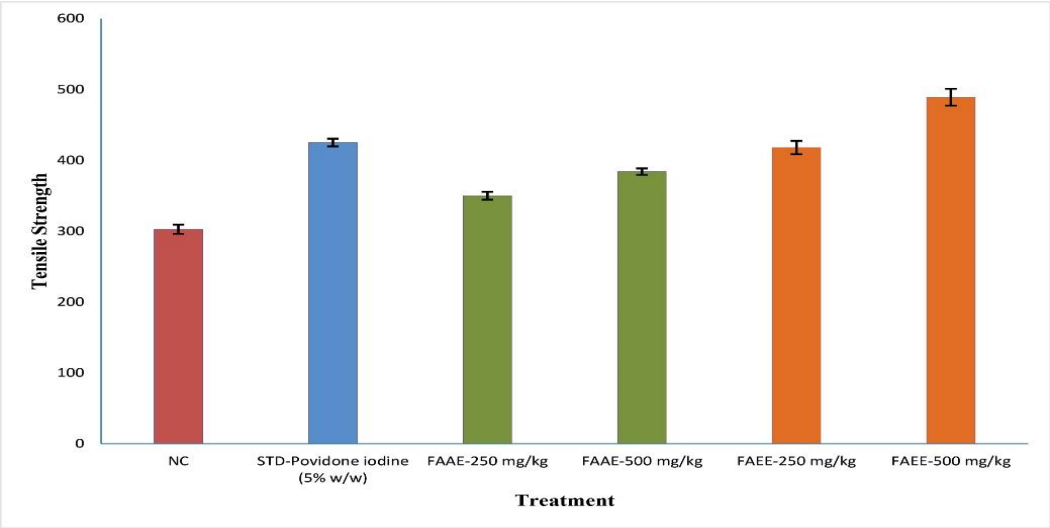

Fig No. 5: Effect of *Ficus retusa* stem bark extracts on breaking strength in incision wound model

Table 1: Wound-healing effect of *Ficus retusa* in excision wound model

Groups	% Wound Contraction				
	4 Day	8 Day	12 Day	16 Day	Epithelization Time (Days)
NC	21.3±0.30	62.25± 0.68	72.4±0.56	88.8±0.72	24.0±0.87
5% w/w-STD	36.0±0.54*	78.8±0.39**	96.0±0.61**	102.0±0.74**	18.02±0.28**
5% FAAE	24.0±0.47	63.2±0.84**	77.9±0.52**	91.8±0.70*	22.8±0.34*
10% FAAE	32.4±0.58**	72.6±0.48**	86.8±0.43**	95.4±0.64**	20.6±0.34**
5% FAEE	35.2±0.78**	76.5±0.54**	90.6±0.54**	98.8±0.35**	22.0±0.26**
10% FAEE	41.2±0.69*	79.0±0.62*	94.8±0.56*	01.5±0.94**	19.2±0.58**

*NC= Normal control, STD (Standard drug) = Povidone iodine, FAAE= *Ficus retusa* acetone extract, FAEE= *Ficus retusa* ethanolic extract

Table 2: **Wound-healing effect of *Ficus retusa* in incision wound model**

Group	Tensile Strength (g)
NC	302.7±6.47
STD-Povidone iodine (5% w/w)	425.0±5.36**
FAAE-250 mg/kg	350.0±5.51**
FAAE-500 mg/kg	384.0±4.56**
FAEE-250 mg/kg	418.0±9.37**
FAEE-500 mg/kg	489±11.85**

*NC= Normal control, STD (Standard drug) = Povidone iodine, FAAE= *Ficus retusa* acetone extract, FAEE= *Ficus retusa* ethanolic extract

DISCUSSION

Inflammation, granulation tissue development, and extracellular matrix remodelling are the three overlapping phases of wound healing, which is a highly complicated yet carefully planned series of events[10]. Numerous cellular processes are involved in these events, including adhesion, migration, proliferation, and phenotypic differentiation. The first stages of wound healing include inflammation and the manufacture of ground material, and clot formation occurs immediately following damage[11]. The majority of the extracellular matrix's heterogeneous, nonfibrillar components, known as proteoglycans, make up the ground material. These intricate macromolecules consist of linear heteropolysaccharides called glycosaminoglycans (GAGS), which are covalently bonded to a protein core[12]. It has been demonstrated that proteoglycans and GAGS are crucial to each of the aforementioned wound-healing processes[13]. The present research sought to determine if giving *F. retusa* ointment to rats hastened wound healing. The outcomes of this investigation demonstrated that both excision and incision control animals had slower wound healing. Furthermore, ethanolic extract treatment dramatically increased the wound healing rate and reduced the time required to repair wounds. These data show that *F. retusa* increased the speed at which rats' wounds healed. *F. retusa* acetone and ethanolic extracts were tested for safety using the acute toxicity assay. The extract was determined to be safe at a dosage of 2000mg/kg; 14 days later, the animals exhibited no evidence of skin response, irritation, redness, inflammation, or erythema.

CONCLUSION

The phytochemical analysis of acetone and ethanolic extracts revealed the presence of flavonoids, tannins, alkaloids, carbohydrates, glycosides, and steroids. The current study investigated acute toxicity up to a dosage of 500mg/kg. At this dosage, animals exhibited hypersensitivity, grooming, restlessness, irritability, itching, CNS excitement, increased muscular tone, and piloerection. Results demonstrate that the acetone and ethanolic extracts of *F. retusa* stem bark possess better wound healing potency, which was evidenced by the increased rate of wound contraction, reduction in the period of epithelization, increase in collagen deposition, and increase in tensile strength in granulation tissues.

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***Address for correspondence**
Puneet Bhardwaj
Lords International College of Pharmacy, Alwar, Rajasthan.
Email:
puneetbhardwaj1022@gmail.com