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RESEARCH ARTICLE

WOUND HEALING ACTIVITY OF METHANOLIC LEAF EXTRACT OF *Ficus carica* IN ALBINO RATS

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ABSTRACT

The entire wound healing process is a complex series of events that begins at the moment of injury and can continue for months to years. The stages of wound healing are inflammatory phase, proliferation phase, fibroblastic phase and maturation phase. Several Investigators has been found that, most of the tribal people are using *Ficus carica* for wound healing, activity apart from in other conditions. So in the present study emphasis will be laid on the pharmacological screening of the plant with special reference to the wound healing activities. Methanolic leaf extract of a *Ficus carica* having significant wound healing activity in albino rats in excision wound model.

INTRODUCTION

Normal wound healing response begins the moment the tissue is injured. 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 begin to increase collagen production. Later, the epithelial tissue is regenerated (Souba 1999). Many Ayurvedic plants have a very important role in the process of wound healing. Plants are more potent healers because they promote the repair mechanisms in the natural way (Chitra shenoy 2009). Plant derived compounds have been a part of the evolution of human, healthcare for thousands of years. Today a substantial number of drugs are developed from plants which are active against a number of diseases. Plant based medicines have been the basis of treatment and cure for various diseases and physiological conditions in traditional methods practiced. Metabolites or bioactive compounds from plants play an important role in conventional as well as western medicine. Indian medicinal plants also provide a rich source for antioxidants that are known to prevent/delay different diseased states. The antioxidant protection is observed at different levels (Baie saringat 2000). Medicinal plants have been used since time immemorial for treatment of various ailments of skin and dermatological disorders wounds and burns (Saha 1997). *Ficus carica* family - Moraceae constituted one of the largest genera of medicinal plants with about 750 species of woody plants, trees, and shrubs primarily occurring in subtropical and tropical regions through out the world. It is commonly call as anjir (Hindi) or fig fruit in English. It is a very nourishing food and used in industrial products. It is rich in vitamins, mineral elements, water, and fats. Figs are one of the highest plant sources of calcium. *F. carica* has been reported to include antioxidant, antiviral, antibacterial, antiinflammation, haemostatic, hypoglycemic, hypocholesterolaemic, cancer suppressive and anthelmintic effects (Canal 2000, Gilani 2008, Jeong 2009, Perez 1999). *Ficus carica* is a dry fruit is also considered a good nutritional support for diabetics. These compounds bring styptic effect through its astringent action in controlling menorrhagia. *Ficus carica* Linn. is used in the formulation of 'Stone crush', as a daily health supplement by keeping the urinary tract flushed, urolithiasis, crystal urea, burning following lithotripsy and urinary tract infections. Apart from all these it also contains antimicrobial activity due to presence of psoralin and bergaptin. So in the present study emphasis will be laid on the pharmacological screening of the plant with special reference to the above mentioned activities. The objectives of the study are to screen wound healing activity on influence of extract of *Ficus carica* in wound healing and to screen drugs that promote healing. Several materials have so far been used and are reported to affect healing differently however, intensive research in wound healing has not yielded, economic and efficacious pro-healing agent that could obviate the long hospitalization of patients following surgery and wound infliction.

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MATERIALS AND METHODS

Plant material

Plant material of *F. carica* leaves were collected from Madikonda, Warangal, Andhra Pradesh, India and authenticated by Prof. M.A.Singara Charya, Kakatiya University, Warangal.

Preparation of Extraction

The leaves were carefully dried in shade for 15 days. To ensure complete dryness kept in hot air oven at 45˚C for 5 minutes. Then leaves were subjected to size reduction to make powder. The dried and powdered leaves were subjected to hot extraction in Soxhlet apparatus. The powered plant material were subjected to Soxhlet extraction with methanol (50%) at 70^0c, this extract was then

concentrated by evaporation of solvent through hot air oven under controlled temperature (50^0c) for 24hrs to evaporate the methanol from it. A dark brown colored residue was obtained, extract were stored at $4C^o$ until it is used. Preliminary photochemical screening (Chitra shenoy 2009) were carried out for the presence or absence of phyto constituents like Glycosides, Flavanoids, Saponins, Alkaloids, Carbohydrates, Sterols, Proteins, Phenolic compounds and Reducing compounds (Harbone JB).

Antibacterial activity

Muller Hinton Agar was prepared according to the manufacturer's instructions. The medium was sterilized by autoclaving at 121°C for 15 minutes at 15 psi pressure and was used to determine the antibacterial activity of *ficus carica* methanolic leaf extract. Sterile molten cool (45°C) agar was poured aseptically into sterile Petri plates (15 ml each) and the plates were allowed to solidify at room temperature in sterile condition. After solidification and drying, the plates were seeded with appropriate micro organisms by streaking evenly on to the surface of the medium with a sterile spreader and wells (6 mm diameter) were cut out from the agar plates using a sterile stainless steel bore and filled with 60 µl of the each extract solution in respective wells. Streptomycin was used as standard control respectively. Then the plates were incubated at 37°C for 24 hrs in the next day the zones of inhibition were measured with a measuring scale. This experiment was carried out in triplicate for their confirmation. The results were read by the presence or absence of zone of inhibition.

Skin irritation studies

The rabbits are shaved the skin in two different position of dorsal side, about $300mm^2$, rabbits were kept under rabbit holder and apply ointment on one side and other side serve as control.

Wound-healing Activity

Excision wound models were used to evaluate the wound-healing activity of methanolic leaf extracts of *ficus carica.*

Chemicals

Framycetin, Wool fat, Hard Paraffin, Cetostearyl alcohol and White Soft Paraffin.

Wound Healing Activity Tests Animals

Wistar albino rats weighing about (150–250g) were used for the study. They were fed with standard chow. They were housed in polypropylene cages maintained under standard conditions (12 hour light - dark cycle; 25 ± 3 °C; 35–60% humidity). The animals were left for 3 days at room conditions for acclimatization. They were maintained on standard pellet diet and water ad libitum throughout the experiment. A minimum of six animals were used in each group. The study was permitted by the Institutional Animal Ethics Committee and was performed according to the international rules relating to animal experiments.

Preparation of ointment by fusion method

(a) Preparation of simple ointment: Wool fat – 2.5 gm: Hard Paraffin-2.5 gm; Cetostearyl alcohol -2.5 gm; White Soft Paraffin-42.5 gm. Each ingredient was mixed and heated gently with stirring then cooled. The base was then packed in a wide mouth container.
(b) Preparation of 10% ointment: 5 gm methanol leaf extract of *ficus carica* was added slowly to the above melted ingredients and stirred thoroughly until the mass cools down and a homogeneous product is formed. The ointment was then packed in a wide mouth container.

Excision Wound Model

The Excision model was used to check the wound contraction and wound closure durable time. Each group of six animals was anaesthetized under light ether anesthesia. The hair on the back of the rat was removed by shaving with an aseptic surgical blade. A circular wound was created (Morton and Melon 1972) on the dorsal interscapular region of each animal by excising the skin with a 300 mm^2 biopsy punch; the wounds were left open. The base and *ficus carica* leaf extracts ointments and 2% framycetin standard ointment were applied topically once a day on the wound till they completely healed. The progressive changes in wound area were monitored plan metrically by tracing the wound margin on graph paper every alternate day. Epithelialisation time was noted as a number of days after wounding required for the scar to fall off leaving no raw wound behind. From the healed wound, a specimen sample of tissue is isolated from each group of rats for histopathological examination (Jeong 2009).

Histopathological studies of excision wound

Sample tissues were fixed in 10% formalin and embedded in paraffin wax. Serial sections (5 µm thickness) of paraffin-embedded tissues were cut. The tissues were stained with haematoxylin and eosin, which were examined by light microscope (Galighor 1976) (Luna GLHT.1968). Percentage of wound contraction is evaluated in the skin tissues.

Statistical analysis

The values were calculated as mean ± S.E.M. The significance of the difference of the mean value with respect to control group was analyzed by one way ANOVA followed by Dunnet's t- test using statistica 8.0. Statistically significant at a level of $P<0.05$ or above was considered to be significant.

RESULTS AND DISCUSSION

Phytochemical screening

The preliminary phytochemical screening of methanolic leaf extracts of *ficus carica* were evaluated for the presence of alkaloids, glycosides, steroids, fixed oils, fats, terpenes, tannins and flavonoids. The results revealed that the presence of Steroids flavonoids, whereas Alkaloids, Carbohydrates, Glycosides, reducing sugars, Tannins are not seen in methanolic leaf extract.

Table 1. Preliminary screening of phytochemicals in *Ficus carica* leaf extract

Test	Conformation
alkaloids:	-ve
Carbohydrates	-ve
Glycosides	-ve
reducing sugars	-ve
Steroids	+ve
Tannins	-ve
Flavonoids	+ve

Antibacterial activity

Determination of antibacterial activity by agar well diffusion assay showed that methanolic extract of *F. carica* leaves exhibited the antibacterial effect against pathogenic bacteria was evaluated for its antibacterial activity using the cup plate method with framycetin as reference standard. The antibacterial studies revealed that the methonolic leaf extract has antibacterial activity against both gram positive and negative bacteria. The standard framycetin showed the all most same level of inhibition against *Escherichia coli*. The inhibition zone around each well amended with Leaf extract

or framycetin reveals that the highest antimicrobial activity was observed against *Escherichia coli* (22 mm), followed by *Salmonella paratyphi* (20 mm). The lowest activity levels were observed against *Proteus vulgaris* and *Staphylococcus aureus* (8 mm each). The antibacterial activity reveals that the produced extract has a broad spectrum activity against gram positive and gram negative bacteria. (Table 2).

Table 2. Effect of methanolic leaf extract of *ficus carica* on different micro organisums

S.No	Test Organisms	Leaf Extract	Standard
1	*Klebsiella pneumoniae*	12 mm	26mm
2	*Proteus vulgaris*	06 mm	14 mm
3	*Salmonella paratyphi*	20 mm	24 mm
4	*Staphylococcus aureus*	08 mm	12 mm
5	*Pseudomonas aeruginosa*	10 mm	12 mm
6	*Bacillus megaterium*	08 mm	13 mm
7	*Bacillus stearothermophilus*	14 mm	19 mm
8	*Escherichia coli*	22 mm	24 mm
9	*Bacillus subtilis*	12 mm	19 mm
10	*Bacillus cereus*	14 mm	20 mm

Skin irritation test

When 10% *ficus carica* methanolic leaf extract ointment is applied to rat skin and observe, there is no noticeable redness and inflammation. The extract is free from irritation.

significant wound healing is observe from sixth day onwards

Wound healing is a process by which a damaged tissue is restored as closely as possible to its normal state and wound contraction is the process of shrinkage of area of the wound. It mainly depends on the repairing ability of the tissue, type and extent of damage and general state of the health of the tissue. The granulation tissue of the wound is primarily composed of fibroblast, collagen, edema, and small new blood vessels. The undifferentiated cells of the wound margin modulate themselves into fibroblast, which start migrating into the wound gap along with the fibrin strands. The collagen composed of amino acid is the major component of extra cellular tissue, which gives strength and support. The wound concentration used for the measuring the excision wound model. The wound induced by excision wound model as shown (Fig-1) in rat and applied ointment regularly two times a day.

Fig. I. Photographic representation of excision wound in rat

The *Ficus carica* methanolic leaf extract exhibited significant wound healing activity as compared to control in excision wound model. It is observed that the wound contracting ability of the 10% (w/w) extract ointment treated groups showed significant wound healing from third day on wards. The wound closure time was lesser as well as the percentage of wound contraction was more with the 10 % (w/w) extract ointment treated group. The percentage of wound contraction with extract ointment treated group was found to be earlier as compared to control. 100% wound contraction is observe in test group (Fig: II). The wound were completely healed in treated group (epithelisation period) 14±2 days where as in the control animals 24±2days. In standard group 12±2 days (Table: III) The multiple sections studied in histopathological examination of the tissues of the

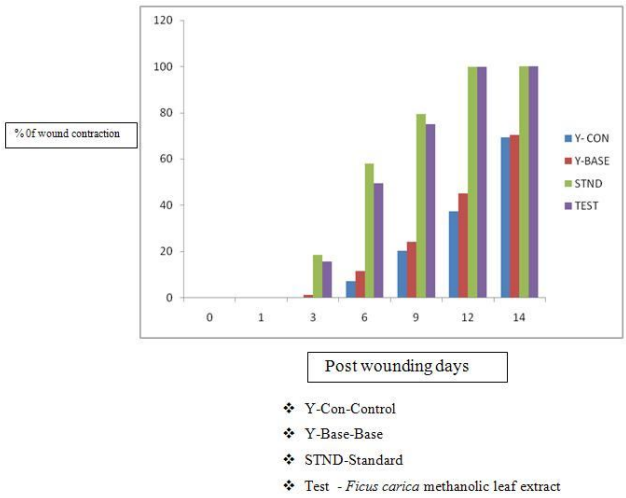

Fig. II. Effects of *ficus carica* and Framycetin on excision wound model in all groups of animals

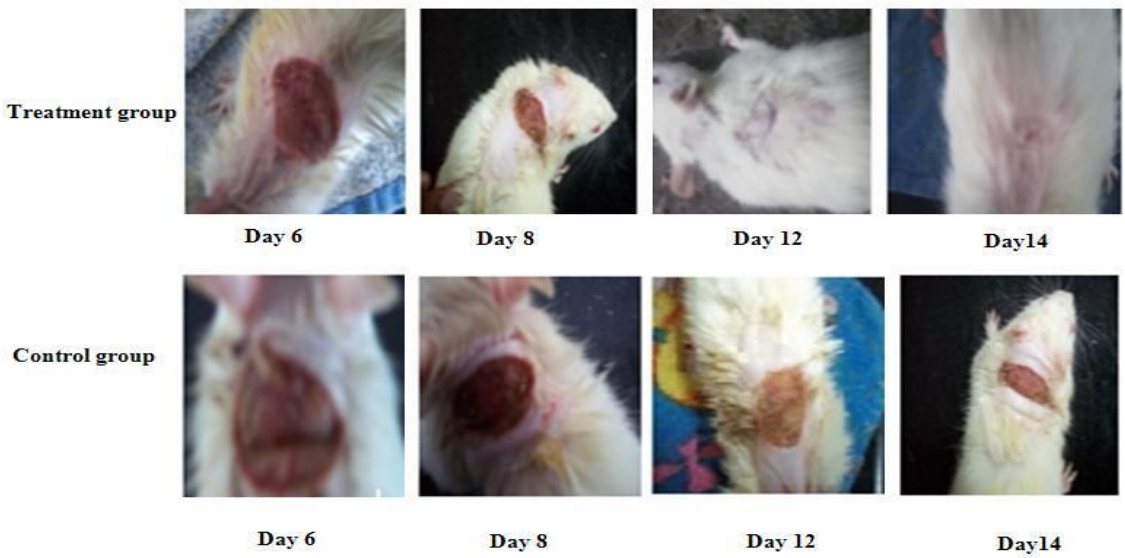

Fig. III.Photographical representation of contraction rate on different days

Table 3. Effect of methanolic leaf extract of *ficus carica* on skin irritation study

group	Sign	Score
Control	-	0
10%f *ficus carica* leaf extract ointment	Not noticeable redness and inflammation	0

Table 4. Effect of *ficus carica* methanolic leaf extract on wound healing by excision wound method in albino rats

Post Wounding Days	Wound area (mm2) mean± SEM) and percentage of wound contraction			
	Control	Base	Framycetin	*Ficus carica* extract
0	235±0.2 (0.00%)	235±0.42 (0.00%)	235±0.5 (0.00%)	235±0.45 (0.00%)
1	235±0.4 (0.00%)	235±0.25 (0.00%)	235±0.35 (0.12%)	235±0.38 (0.01%)
3	235±0.25 (0.00%)	233±0.28 (1.03%)	195±0.33 (18.3%)	200±0.25 (15.5%)
6	220±0.32 (7.02%)	210±0.32 (11.42%)	100±0.36* (58.04%)	120±0.34* (49.4%)
9	190±0.45 (20.2%)	180±0.25 (24.05%)	50±0.22** (79.32%)	60±0.22** (75.02%)
12	150±0.35 (37.3%)	140±0.28 (45.03%)	10±0.44** (99.9%)**	13±0.2** (99.84%)**
14	75±0.20 (69.2%)	70±0.22 (70.4%)	0 (100%)	0 (100%)

Each value is the mean ± S.E.M. of six rats.
* P<0.05,**P<0.01Vs controle, one way ANOVA followed by Dunnet's t-test.

phytoconstituents belongs to different plants Curcuma (turmeric), *Allium sativum* (garlic), *Allium cepa* (onion), and *P.zeylanicum*, Henna, Peru etc. (Robak et al1988) (Bairy KL 2002) are effective wound healing activity. The phytochemical screening of *Ficus carica* reveals the presence of steroids and flavonoids in the methanolic extract. The flavonoids are responsible for wound healing activity is observed in this study; however, further phytochemical studies are needed to isolate the active compound responsible for these pharmacological activities. The skin irritation study on rabbit skin prove that the drug does not show any type of inflammation when applied on skin (Table-2). This suggests that the *ficus carica* contain some chemical constituents which did not produce any inflammation but increase wound healing activity. Determination of antibacterial activity by agar well diffusion assay showed that methanolic extract of *F. carica* leaves exhibited the antibacterial effect against pathogenic bacteria was evaluated for its antibacterial activity using the cup plate method with framycetin as reference standard. The inhibition zone around each well amended with Leaf extract or framycetin reveals that the highest antimicrobial activity was observed against *Escherichia coli* (22 mm). The lowest activity levels were observed against *Proteus vulgaris* and *Staphylococcus aureus* (8 mm each). The antibacterial studies revealed that the methonolic leaf extract has antibacterial activity against both gram positive and negative bacteria. The topical application of drugs is an efficient therapy method of destroying microbial populations because the availability of the drug at the infected wound site leads to enhanced wound healing activity. The virulence capacity of micro-organisms and host immune response are important factors that can cause massive damage during infection. Normally, common wound

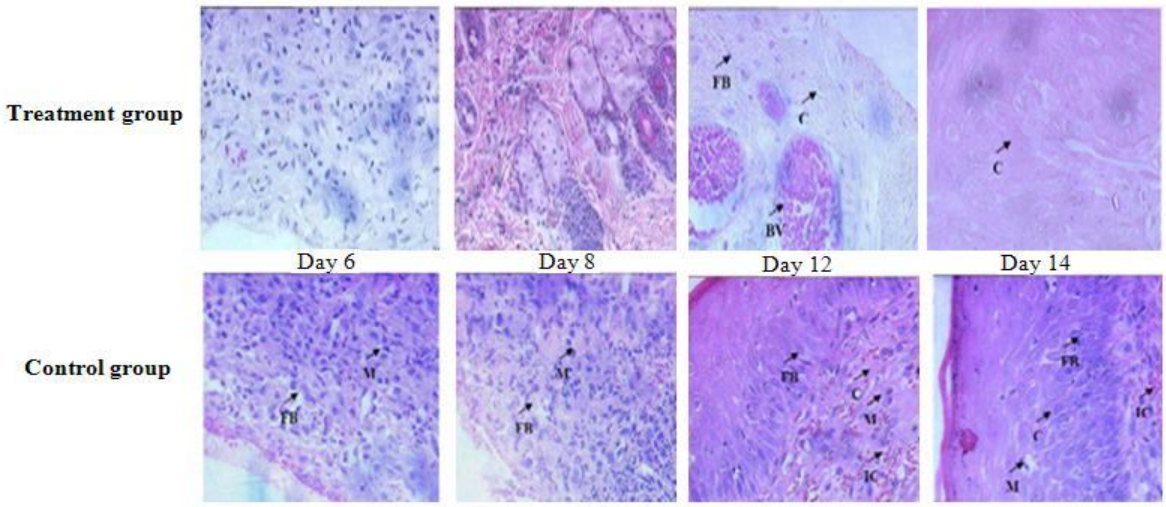

Fig. IV. Hematoxylin and eosin stained sections of the granulation tissue in treated group and at different time intervals. Fibroblasts (FB), collagen (c), vascularisation control group with larger blood vessels (BV) and inflammatory cells(IC).S

wound area treated with extract ointments (10% w/w), 2% w/w framycetin ointment and simple ointment treated groups were shown in (Fig: IV). The histological examination showed that the original tissue regeneration was much greater in the skin wound treated with extract ointments and framycetin ointment treated group without any edema, congestion, or inflammatory changes. In the control group it was partially tissue regeneration and necrotic tissues were not completely replaced by granulation tissues. Collagen fibers were abnormally thickened and disordered in the wound.

DISCUSSION

The present investigation describes some unique features of the leaves extract from the plant of *Ficus carica* with respect to its potential wound healing capacity in rats. Plant products are potential wound healing agents, and largely preferred because of their widespread availability, non-toxic, absence of unwanted side effects, and effectiveness as crude preparation. Earlier it was report that the pathogens such as *S.aureus*, *C.albicans*, and *P.aeruginosa*. After injury, revascularization of the wound bed and redevelopment of the extracellular matrix are achieved through cell proliferation and the production of granulation tissue. Wound contraction, a part of the proliferative phase of wound healing, occurs through the centripetal movement of the tissues surrounding the wound, which is mediated by myofibroblasts. In present study wound is induced by excision wound model (Fig-1).The increased wound contraction in the treated group may be due to the enhanced activity of fibroblasts and successful elimination of yeast by the *Ficus carica* leaf extract. The slow rate of wound closure in the control group might be attributed to the presence of micro-organisms and their metabolites, which inhibit wound contraction and deteriorates the wound healing activity. A significant increase in collagen content due to enhanced migration of fibroblasts and epithelial cells to the wound site was observed during the wound healing process in the treated group and standard group (Figure-III). 100% wound healing with extract ointment is observe (Fig-II).Complete wound healing is observe with *ficus carica*

leaf extract with in 14±2 days than compare with control group is 24±2 days. A close examination of granulation tissue sections revealed that tissue regeneration was much quicker in the treated group compared to that in control wounds. Increased cellular infiltration observed from hematoxylin and eosin staining in both groups may be due to the presence of pathogens, but the antimicrobial property of *Ficus carica* massively reduced the bacterial population, thereby indirectly reducing the inflammatory cells on the wound site. Early dermal and epidermal regeneration in the treated group (Fig-IV) confirmed that the ointment containing the *Ficus carica* extract had a positive effect toward cellular proliferation, granulation tissue formation, and epithelialization. Incomplete epithelialization with less extracellular matrix synthesis was observed in control rats. Clumps of degenerating neutrophils, necrotic changes, and the persistence of inflammatory exudates in the upper dermis with loss of epidermis were also observed up to day 8. The treated rates showed marked epithelialization, a moderate amount of extracellular matrix synthesis, and new blood vessel formation.

Conclusion

The results obtained in the present study clearly indicate that the Methanolic extract of leaves of *ficus carica* are having significant wound healing activity in rats when apply topically on wound. Flavonoids, steroids are known to be having active antibiotic principles. The wound healing effect of ethonolic extract of leaves of *ficus carica* may be due to the presence of more than one active principle mentioned above.

REFERENCES

Baie Saringat, H.J. Sheikh KA. 2000. The wound healing properties of *Channa striatus-cetrimide* cream-tensile strength measurement. *J Ethanopharmocol*. 71, 93–100.

Bairy, K.L, 2002 .wound healing potential of some plant products *Nat Remed* 2, 11.

Canal, J. R., M. D. Torres, A. Romero, and C. A. Perez. 2000. Chloroform extract obtained from a decoction of *Ficus carica* leaves improves the cholesterolaemic status of rats with streptozotocin-induced diabetes. *Acta Physiologica. Hungarica* 87, 71-76.

Chitra shenoy, M.B. Patil, Ravikumar 2009. Preliminary phytochemical investigation and wound healing activity of *Allium.Cepalin* (Liliaceae). *International journal of pharmacy and pharmaceutical sciences*. 2, 2.

Galighor, A.E. Kozloff, E.N., 1976. In: Essentials of practical Micro Technique. 2nd Ed. New York: Lea and Febiger,

Gilani, A. H., M. H. Mehmood, K. H. Janbaz, A. U. Khan, and S. A. Saeed. 2008. Ethnopharmacological studies on antispasmodic and antiplatelet activities of *Ficus carica*. *J. Ethnopharmacol*.119:1-5.

Govindarajan, R. Madhavan, V. Rao, C.V. Shirwaikar, A. Mehrotra, S. Pushpagandan, P. 2004. Healing Potential of Anogeissus latifolia for dermal wounds in rats. *Acta Pharm*.54, 331–8.

Jeong, M. R., Kim, H. Y. and Cha, J. D. 2009. Antimicrobial activity of methanol extract from *Ficus carica* leaves against oral bacteria. *J. Bacteriol. Virol*. 39, 97-102.

Lodhi, S. Pawar, R. S. Jain, A. P. and Singhai, A. K. 2006. "Wound healing potential of *Tephrosia purpurea* (Linn.) Pers. in rats," *Journal of Ethnopharmacology*. 108, 2, 204–210.

Luna G.L.H.T. 1968. Manual of Histologic and special staining methods of the Armed Forces Institute of Pathology. 3rd Ed. New York: Mc Graw Hill.

Perez, C., Canal, J. R., Campillo, J. E. Romero, A. and Torres, M. D. 1999. Hypotriglyceridaemic activity of *Ficus carica* leaves in experimentalhypertriglyceridaemicrats.*Phytother.Res*.13,188-191.

Robak, J. and Glyglewski .R.J, 1988. Evolution of wound healing activity of selected traditional medicinal plants from peru, *Biochem Pharmacol* 37,837-841.

Rozaini, M.Z. Zuki, A.B.Z. Noordin, M. Norimah, Y.Hakim, A.N. 2004. The effects of different types of honey on tensile strength evaluation of burn wound tissue healing. *Intern J Appl Res* V et Med. 2, 2.

Saha, K. P. K. Mukherjee, Das, J. Pal, M. and B. P. Saha, 1997. "Wound healing activity of Leucas lavandulaefolia Rees," *Journal of Ethnopharmacology*. 56, 139–144.

Souba WW, Wilmore D. 1999. Diet and nutrition in case of the patient with surgery, Trauma and sepsis. In Modern Nutrition in Health and Disease. 9, 1589-1618.

Swamy H. M. K., V. Krishna, K. Shankarmurthy, *et al*., 2007. "Wound healing activity of embelin isolated from ethanol extract of leaves of Embelia ribes Burm," *Journal of Ethnopharmacology*, vol. 109., 529–534.

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