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**EFFECTIVENESS OF TOPICAL APPLICATION OF
 CRUDE EXTRACT OF *ICHNOCARPUS FRUTESCENS* TO WOUNDS –
 A PROSPECTIVE OBSERVATIONAL STUDY**

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Abstract

The methanolic extract of *Ichnocarpus frutescens* was evaluated for its wound healing potential by using incision and excision models. The effects of the test drug on the rat for wound healing were assessed by the rate of wound closure, period of epithelialization, tensile strength and histopathology of granulation tissue. The results signify the wound healing activity of *Ichnocarpus frutescens*.

Keywords: *Ichnocarpus frutescens*, Incision, Excision, Wound healing, Tensile strength.

Introduction

Wound healing is a physiological response that results in the restoration of anatomic continuity and function. Cutaneous wound healing encompasses multiple overlapping events following injury, including coagulation, leukocyte recruitment, matrix deposition, epithelialization and resolution of inflammation with formation of a mature scar. Medicinal plants have greater potentials and show beneficial usage in wound care, preventing the rate of wound healing with minimal pain, discomfort and caring to the patient. Therefore, the present study was conceptualized to investigate the effect of *Ichnocarpus frutescens* on wound healing.

Ichnocarpus frutescens R. Br., commonly known as Black Sariva, is an important medicinal plant

found through out the India, belonging to family Apocyanaceae¹. *Ichnocarpus frutescens* is used in the indigenous system of medicine in the treatment of fevers, gout, rheumatism, arthritis, epilepsy, venereal diseases, herpes and skin diseases^{2,3,5}. Studies on chemical constituents of the plant indicated the presence of the pharmacologically active constituents like phenylpropanoids, phenolic acids, coumarines, iridoid glycosides, flavonoids, sterols and pentacyclic triterpenoids⁴. Pharmacological study revealed hepatoprotective, antioxidant and anti-inflammatory analgesic, antidiabetic and antitumour activity^{6,7,8,9}.

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Materials & Methods

Plant material: *Ichnocarpus frutescens* aerial parts were collected during the month of June from Kancheepuram district, Tamilnadu, India and authenticated by Dr. S Emmanuel sj, Botanist, Loyola Academy, Alwal. A voucher specimen has been deposited in the herbarium of the Department of Agriculture Science, Loyola Academy Degree & PG College, Alwal, India, for future reference.

Experimental Animals

Male wistar rats (150- 180g) were purchased from the animal house of Mahaveera Enterprises, Hyderabad, India. The experimental protocol was approved by the Institutional Animal's Ethics Committee and by the regulatory body of the government (Regular No 1221/a/08/CPCSEA).

The animals were reared for three days at room conditions for acclimatization. Rats were housed in colony cages (4 rats per cage), at an ambient temperature of 25±2°C and 45-55% RH, with a 12 hr light/12 hr dark cycle. They had free access to standard pellet chow (Hindustan lever Ltd., Bangalore, India) and water *ad libitum*. The animals were housed 4 per cage in polypropylene cages provided with paddy husk as bedding for 7 days at room conditions for acclimatization. Prior to each study, the animals were made to fast for 12-14 hr but had free access to water.

Experimental design

Rats were divided into four groups of four animals each for wound healing models.

Group – I (Control group) rats with excised and incised wounds were treated with soft white petroleum jelly. Group – II (Test group one) was treated with MEIF (10% w/w) in soft white petroleum jelly applied locally in excised and incised wound models. Group – III (Test group two) was treated with EAEIF (10 % w/w) in soft white petroleum jelly applied locally in excised and incised wound models. Group – IV (Standard group) treated with Soframycin (1% w/w) ointment applied locally on excised and incised wounds.

Preparation of plant extract

The leaves were dried under shade, powdered and passed through (60-120 meshes) and stored in closed vessel for further use. The dried powder (4 kg) was successively extracted with organic solvent in vacuum under pressure using rotary flash

evaporator by using different solvent like methanol and ethyl acetate.

Phytochemical Screening

Standard screening tests of the extracts were carried out for various plant constituents. The crude extracts were screened for the presence or absence of secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins, tannins and anthraquinones using standard procedures.

Experimental procedure

Excision and incision wounds were created in rats under the anesthesia induced by diethyl ether (45 mg/kg bw ip). The extract was applied locally once daily on the excision and incision wounds. In case of incisional wound, the treatment was continued till 10th day post wounding, while in case of excisional wound it was continued till 16th day post wounding.

Incisional wound model

A 5cm linear para vertebral incision was made with a sterile surgical blade through the full thickness of the skin at the distance of 1.5 cm from the midline of each side of the depilated back of the rat^{10,11}. The wounds were closed with three surgical interrupted sutures of 0.5cm apart. All the drugs were given daily till epithelialization. Sutures were removed on the 11th post wound day. On 1st, 4th, 8th, 12th and 16th day the animals were sacrificed for histopathological studies.

Excision wound model

An impression was made on dorsal thoracic region 1 cm away from vertebral column and 5cm away from ear using a round seal of 2.5 cm diameter on the anaesthetized rat.¹¹ The skin of impressed area was excised to full thickness to obtain a wound area of about 500mm diameter. Homeostasis was achieved by blotting the wound with cotton swab soaked in normal saline. Wound contraction was studied by tracing raw wound area on day 1, 4, 8, 12, & 16 on graph paper. The degree of wound healing was calculated¹².

Wound contraction

On 1st, 4th, 8th, 12th and 16th day, after wound creation, four animals in each group (group I to IV) were sacrificed randomly. The wound contraction was monitored by measuring the progressive

changes in raw wound area planimetrically on a transparent paper¹³. The tracing was then transferred to 1mm² graph sheet, from which the wound surface area was calculated. The evaluated

surface area was then employed to calculate the percentage of wound contraction, taking the initial size of the wound as 100% by using following formula.

$$\text{Wound contraction \%} = (\text{original wound area} - \text{specific day wound area}) / \text{original wound area} \times 100$$

Tensile Strength

The tensile strength of a wound represents the degree of wound healing. Healing tissue along with normal skin at two ends were excised for tensile strength measurement using tensile testing machine TKG-20¹⁴. Strips of 8mm width and 20mm length were cutout from the excised tissue in

treated and control animals and were loaded between the upper and lower holder of the machine in such a way that the effective load bearing size was 8 x 8mm with the wound remaining in the centre. The total breaking load is measured in Newtons and tensile strength was calculated by the following equation

$$\text{Tensile strength} = \text{Total breaking load} / \text{cross sectional area.}$$

Histopathology

At the end of the 16th day samples after treatment were fixed in 10% buffered formalin, processed and blocked with paraffin and then sectioned into 5um thickness and stained with hematoxylin and eosin (HE) stain. They were later observed for histological changes under microscope.

Results

Effect of crude extract on wound contraction (%) and tensile strength (N/cm²) on excision wound model in rats [value are mean \pm SD]

Table No. 01

Wound Contraction					
Treatment	1	4	8	12	16
Control	4.45 \pm 0.03	9.65 \pm 0.02	16.26 \pm 0.03	27.35 \pm 0.03	40.15 \pm 0.03
Methanol Extract	5.46 \pm 0.03	14.26 \pm 0.03	56.46 \pm 0.03	61.96 \pm 0.03	81.16 \pm 0.15
Ethylacetate extract	4.58 \pm 0.08	13.29 \pm 0.49	46.82 \pm 0.4	55.7 \pm 0.58	75.25 \pm 0.44
Soframycin	3.79 \pm 0.05	14.76 \pm 0.04	50.99 \pm 0.17	65.08 \pm 0.25	87.8 \pm 0.29
Tensile Strength					
Treatment	1	4	8	12	16
Control	3.57 \pm 0.16	5.75 \pm 0.1	8.07 \pm 0.2	9.03 \pm 0.31	12.08 \pm 0.34
Methanol Extract	9.67 \pm 0.18	11.73 \pm 0.12	13.67 \pm 0.19	18.6 \pm 0.51	20.93 \pm 0.31
Ethylacetate extract	7.5 \pm 0.08	9.99 \pm 0.28	12.08 \pm 0.36	14.35 \pm 0.67	19.13 \pm 0.41
Soframycin	8.63 \pm 0.25	10.66 \pm 0.35	13.25 \pm 0.3	17.65 \pm 0.22	22.27 \pm 0.65

Table No. 02: Period of Epithelialization

Treatment	Days
Control	20.01 \pm 0.3
Methanol Extract	18.08 \pm 0.11
Ethylacetate extract	16.36 \pm 0.23
Soframycin	15.62 \pm 0.29

The preliminary phytochemical screening of *Ichnocarpus frutescens* extracts showed presence of flavonoids, aminoacids, amino sugars, amines, tannins phenolic substances and proteins respectively in methanolic and ethylacetate extracts. Significant promotion of wound healing was induced by methanolic and ethylacetate extract and is comparable to the reference drug. In the

excision wound model, the methanolic extract treated groups of animals showed 56.46 \pm 0.03% contraction on the wounds at 8th day.

The same extract demonstrated 81.16 (0.15%) contraction on 16th day which was close to contraction value of the reference drug Soframycin. Ethylacetate extract showed 46.82 \pm 0.39% (8th

day) and 75.25 (0.44%) (16th day). However, no significant difference among the treated groups was noticed in the initial periods (Table-1)

Tensile strength of the wounds in animals treated with methanolic extract demonstrated values very close to the reference drug. It was followed by ethylacetate on day 12. Topical application of

methanol and ethylacetate extract on incision wound model demonstrated importance in wound tensile strength compared to control group (Table-2). Wound inflicted on group II and group III were found to epithelize faster in 15 and 16 days respectively as compared to control animals which took 20 days to heal. (Table 2).

Histopathology



Fig. No. 01 : Photographical representation of contraction rate on day 16 in different groups.

Group I	Control Rat
Group II	MEIF
Group III	EAEIF
Group IV	Soframycin

Discussion

The present investigation brings out the potency of the leaf extract from the plant *I. frutescense*, with respect to its wound healing capacity in infected rats. Plant products as wound healing agents are largely preferred because of their wide spread availability, nontoxicity, absence of unwanted side effects, and effectiveness as crude preparations. The preliminary phytochemical investigations reveals the presence of glycosides, saponins, flavonoids and terpenoids in the plant extract.

The constituents of the aerial extracts such as terpenoids and alkaloids may play a major role in the wound healing process observed in the study¹⁵. However, further phytochemical studies are needed to isolate the active compound(s) responsible for these pharmacological activities.

Flavonoids reduce lipid peroxidation by improving vascularity and inhibiting the onset of cell necrosis. Hence, any drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibrils by increasing the strength of collagen fibres

increasing the circulation, preventing the cell damage and by promoting DNA synthesis ¹⁶.

Topical application of *I. frutescens* improved wound contraction and closure and the effects were distinctly visible starting from 4th post wound day. The wound breaking strength is determined by the rate of collagen synthesis and more so by the maturation process where there is covalent binding of collagen fibrils through inter and intra-molecular cross-linking. In the present study, a significant increase in tensile strength on 12th day was

observed in the test group as compared to the control group. Collagen is a major protein of ECM and is the component that ultimately contributes to wounds strength.

It can be concluded from the present findings that *Ichnocarpus frutescens* leaves extract could be efficiently used as a wound healing agent. Wound contraction, increased tensile strength activity support further evaluation of *Ichnocarpus frutescens* in the topical treatment and management of wounds.

Hematoxylin and eosin stained sections of the granulation tissue in treated group

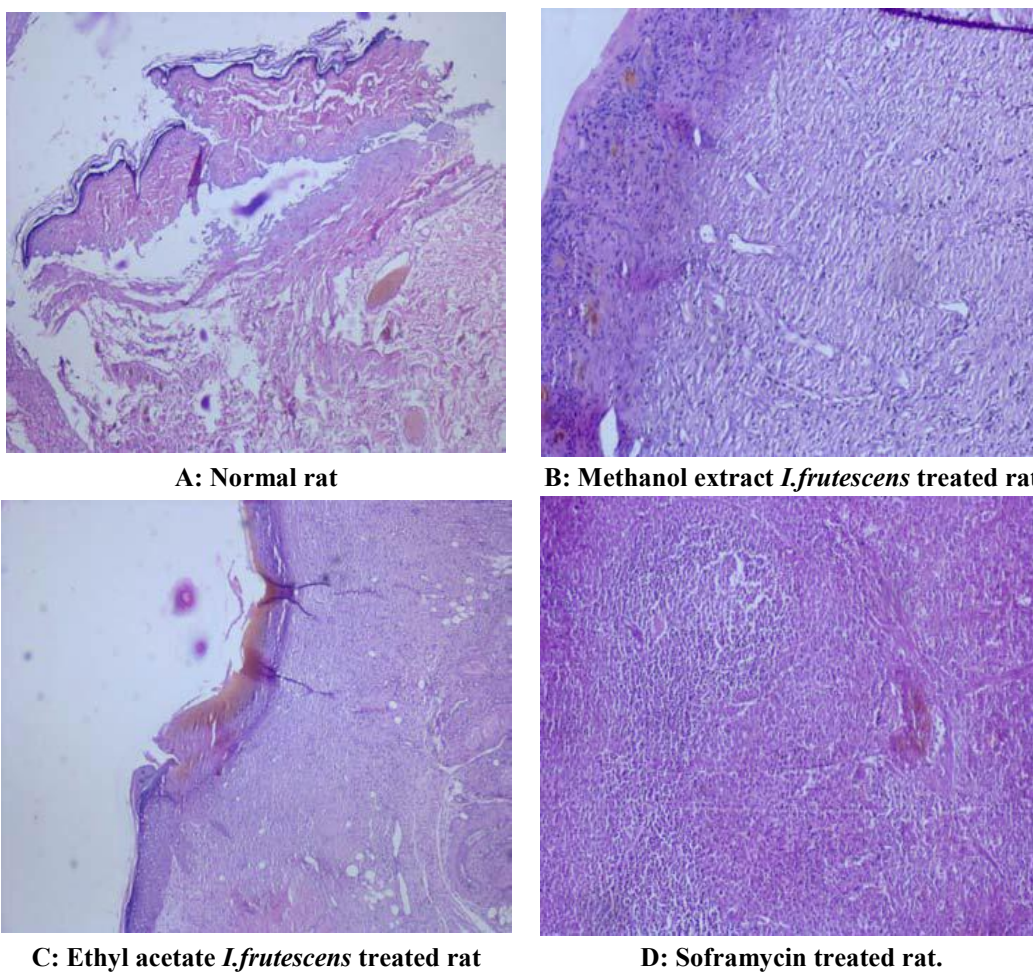


Fig. No. 02

- A. Untreated lesion on day 16 postinjury. Re-epithelialization is well developed but cornification has not yet taken place.
- B. Treated lesion on day 16 post-injury. Re-epithelialization and cornification is well developed. Compared to those of day 10 post-injury the number of blood vessels is reduced. Fewer macrophages and lymphocytes are seen in this section, compared to those of the untreated lesions tissue alignment is improved.

- C. Treated lesion on day 16 post-injury. Compared to those of the untreated lesions a lower number of lymphocytes and macrophages have infiltrated the dermis. Collagen fibers are organizing and the tissue is aligned.
- D. Treated lesion with commercial drug has effected reepithelization and cornification Collagen fibers are organizing and this tissue is aligned.

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