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Research Article

Enhancement of Cutaneous Wound Healing by Ethanolic Extract of *Luffa cylindrica linn.* Leaf and Flower in Wistar rat model.

U. M. Dhanalekshmi, G. Poovi, Narra Kishore, M. D. Raja, P. Neelakanta Reddy*.
Bio - Organic Chemistry Laboratory,
Central Leather Research Institute (Council of Scientific and Industrial Research),
Adyar, Chennai - 600020. India.

ABSTRACT

Aim of study: To explore the wound healing effects of crude ethanolic extract prepared from *Luffa cylindrica Linn.*, leaf and flower.

Material and methods: In a bid to test the wound healing effect of a crude ethanolic extract of Luffa Cylindrica, 24 animals were divided into four groups of six animals each representing control and experimental groups. Each animal had a 2cm x 2cm area of skin on the right dorsolateral flank area marked and excised. The resulting area of skin wound in the experimental group was dressed with crude ethanolic extract while the animals in the control group were dressed with normal saline. The wound area was measured at the fourth, eighth, twelfth; sixteenth post-operative day for animals in both groups and the percentage wound contraction calculated. Sample of granulation tissues and end scar obtained from these animals were used for biochemical and histopathological studies.

Results: The result showed a significant increase in the percentage wound contraction at day 10 in the experimental group compared with the control. The wound of animals in both groups showed excellent granulation tissue formation and minimal signs of wound infection.

Conclusion: It was concluded that flower extract has a better wound healing enhancing action compared with leaf extract. But leaf shows better effect when compared with normal saline treated controls. This study provides a rationale for the topical application of plant extract as a feasible and productive approach to support dermal wound healing.

Key words: *Luffa cylindrica Linn*, leaf , flower , extract , wound healing, biochemical.

INTRODUCTION

Medicinal plants are commonly used for the treatment of various ailments in India, as these are considered to have advantages over the conventionally used drugs that are expensive and known to have harmful side effects (1). Consumption of medicinal herbs is tremendously increasing over a past decade as an alternative approaches to improve the quality of life and maintain a good health. Medicinal plants have been used for centuries as remedies for human diseases (2, 3).

Wound is defined simply as the disruption of the cellular and anatomic continuity of a tissue (4).Wound may be produced by physical, chemical, thermal, microbial or immunological insult to the tissue. The process of wound healing consists of integrated cellular and biochemical events leading to reestablishment of structural and functional integrity with regain of strength of injured tissue. Clinically, one often encounters non-healing, under-healing or over healing. In traditional medicine, extracts of polysaccharide-containing plants are widely employed for the treatment of skin and epithelium wounds and of mucous membrane irritation (5). Medical treatment of wound includes administration of drugs either locally (topical) or systemically (oral or parenteral) in an attempt to aid wound repair (6). The topical agents used include antibiotics and antiseptics, desloughing agents (chemical debridement, e.g. hydrogen peroxide, eusol and collagenase ointment) (7), wound healing promoters and various growth factors (8) necessary for the initiation and promotion of wound healing. Many substances like tissue extracts (9) vitamins & minerals and a number of plant products (10) have been reported by various workers, to possess pro- healing effects. Wound healing herbals encourage blood clotting, fight infection and accelerate the healing of wounds. Plants or chemical entities derived from plants need to be identified and formulated for treatment and management of wounds (11). Several plant genetic resources were tested for their efficacy in healing wounds, namely *Vernonia scorpioides* (12) and *Argemone mexicana (*13).

Like the alchemist's dream of turning base metal into gold, efforts aimed at achieving a perfect wound healing has pushed many researchers into trying various therapeutic options which were thought to aid or accelerate the wound healing process. The cheaper and more effective the agent, the better for the patient. The aim of this study is to evaluate the wound healing effect of a crude ethanolic extract of *Luffa cylindrica* leaf and flower with that of normal saline acting as control.

Luffa cylindrica (LC) belonging to family Cucurbitaceae popularly known as "Raja koshataki", is a traditionally important plant with more medicinal

*Author for correspondence: neelakantareddyp@gmail.com

Table 1. Measurement of wound contraction

SAMPLE	DAY 4	DAY 8	DAY 12	DAY 16
	Wound size (*l x b)*	Wound size *(l x b)*	Wound size *(l x b)*	Wound size *(l x b)*
Control	1.6± 0.033	1.2± 0.021	0.8± 0.023	0.5± 0.022
Leaf extract	1.5± 0.021	1.0± 0.033	0.7± 0.020	0.3± 0.055
Flower extract	1.5± 0.025	0.8± 0.057	0.3± 0.018	healed

Values are mean ± S.E.M. (n = 6)

properties (14). Luffa of Cucurbitaceae has about seven species found in tropical and subtropical regions throughout India, wild in waste lands especially along the coastal area. The climber, *Luffa cylindrica*, known as "peyppirkan" in Tamil is found in the coastal areas of southern India (15, 16). Plants belonging to this family are known to produce a large number of biologically important constituents. Traditionally this plant is used for the treatment of spleenopathy, leprosy, haemorroids, tumours, bronchitis and syphilis (17). The acceptance of traditional medicine as an alternative health care and the development of microbial resistance to the available antibiotics have led researches to investigate the antimicrobial effect of herbal extracts. The potential for developing antimicrobials from higher plants appears rewarding as it may lead to the development of phytomedicine against microbes (18, 19). The present study describes the wound-healing effect of LC leaf and flower extract applied on large full-thickness wounds in the rat.

MATERIALS AND METHODS

Collection of plant material and extraction: The aerial part of the plant was collected from Kanyakumari district of Tamil Nadu, India. It was identified, confirmed and authenticated by comparing with an authentic specimen by a botanist Dr. P. Jayaraman, Plant Anatomy Research Centre, West Tambaram, Chennai. Voucher number of the specimen PARC/2008/154.

The leaf and flower of the plant of *Luffa Cylindrica* was dried in shade and subjected to size reduction separately to get coarse powder. The dried coarse powder of leaf (1kg) and flower (500 g) was successively extracted with hexane, chloroform, ethanol and water using Soxhlet apparatus. The extracts so collected were distilled on a water bath at atmospheric pressure and the last traces of solvent were removed using vacuum. A semisolid crude ethanolic extract alone used for wound healing activity.

Animals

Wistar female albino rats (180- 200 g) used for this study were procured from King Institute Guindy, Chennai, India and housed in the Institutional animal house under standard environmental conditions (23± 1°C, 55± 5 % humidity,12 hours/ 12 hours light/ dark cycle) maintained with free access to standard diet (Hindustan Lever, Bangalore, India) and water *ad libitium*. The 18 animals were divided into three groups, each group containing 6 animals and housed in poly propylene cages. The protocol of animal study was approved by Institutional Animal Ethics Committee (IAEC 03/003/08).

Wound healing experimentation with animals

For the assessment of wound healing activity excision wound model was used. The animals were divided into three groups each group containing 6 animals.

Group I: Control.

Group II: Leaf extract of LC.

Group III: Flower extract of LC.

Production of full- thickness excision wounds

The fur of the dorsum (below the rib cage) of each animal was removed and a full-thickness skin wound was produced on the dorsum of the animal. The animals were anesthetized using diethyl ether before creation of wound and decontaminated by wiping the whole body with sterile antiseptic. The cleared dorsal surface of skin was marked with a sterile square (2×2 cm) stencil. Excising the skin flap in an aseptic environment using sterile scissors and forceps created a full- thickness wound. Each wounded animal was housed in a separate sterile polypropylene cage. Then animals were treated with leaf and flower extract paste. Sterile gauze was reapplied every alternative day. The wound size was calculated and the granulation tissue removed every fourth day.

Measurement of wound contraction

The progression of wound healing can be judged by the periodic assessment of the contraction of excision wounds. Tracing the outline of the wound on tracing sheet and then using graph sheet to

Fig 1. Experimental wound area before and after treatment.

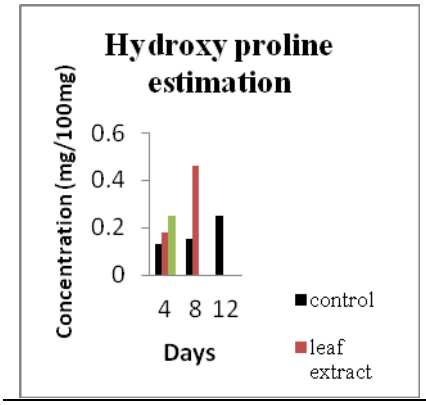

Fig 2. Estimation of hydroxyproline

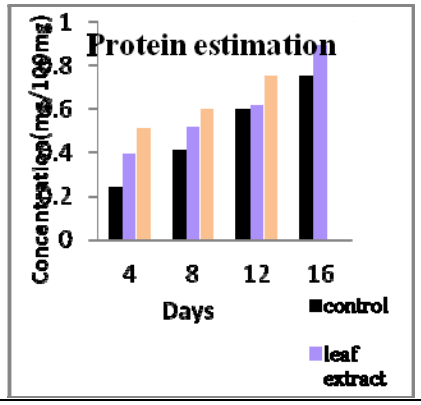

Fig 3. Estimation of protein

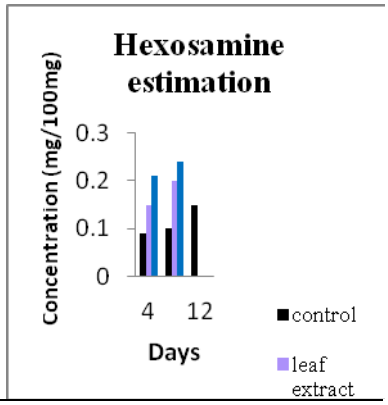

Fig 4. Estimation of hexosamine

calculate the area of the wound size monitored wound contraction. The wound was also pictured. All animals in each group were monitored until complete healing of wounds occurred and the day at which each wound healed was recorded.

Biochemical Analysis

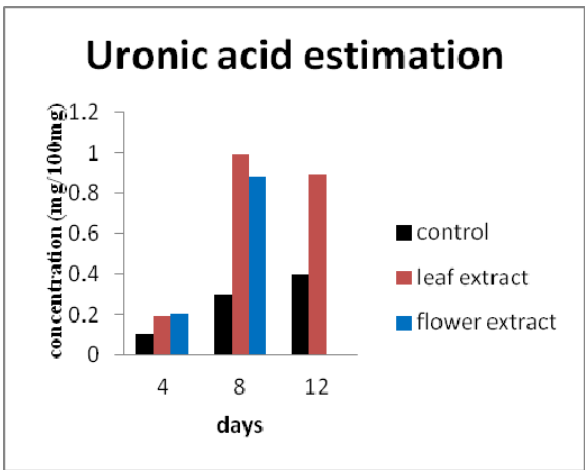

Fig 5. Estimation of uronic acid

All the assays were performed on the granulation tissue collected. Hydroxyproline was measured using the method of Neuman and Logan (20). Total protein was assayed using the method of Lowry (21) using BSA as standard. Hexosamine was estimated by Elson and Morgan (22) and Uronic acid by Bitter and Muir (23).

Tensile Strength

Instron Universal testing machine (450 I) was used for testing material under tension or compression. The test specimen was clamped in the jaws and the machine was run at the rate of 100 ± 2mm/min until the specimen tore apart. The highest load reached was recorded while the sample is subjected to breaking. The distance between the jaws when rupture of the test specimen occurred was noted (24).

Histological Analysis

The granulation tissues were routinely processed by standard procedures and stained with hematoxylin and eosin (H&E). Stained specimens were microscopically evaluated to assess the predominant stages of wound healing.

Statistical Analysis

Data were analyzed and expressed as mean ± S.E.M.

RESULTS

Rate of wound contraction

A better healing pattern with complete wound closure was observed in treated groups within 16 days while it was about 24 days in control rats (Table 1). There was a significant reduction in wound size from day 4 onwards in treated rats and also in later days the closure rate is much faster when compared with control (Fig 1). Visual inspection of the wound showed that all the animals had well-formed granulation tissue. Among the

Table 2.Tensile Strength in the healed tissue of the wounds treated with Saline, Leaf and Flower extract

Sample	Max. load (N)	Max. displacement (mm)	Tensile strength (MPa)	Max. Strain (%)
Control	9.879	5.083	2.470	25.418
Leaf extract	5.896	14.330	0.786	71.650
Flower extract	3.118	16.130	0.520	80.650

experimental groups, animals treated with flower extract showed more significant wound contraction than leaf extract and control groups.

Biochemical Analysis

In the granulation tissue, hydroxyproline and hexosamine level was significantly increased in flower extract treated group when compared with leaf extract treated group and control group (Fig 2 and 3). A highly significant increase in protein content was also observed in the flower extract treated group compared to leaf extract treated and control groups (Fig 4). The Uronic acid content of the flower extract treated group and leaf extract treated group not shown significant difference when compared with control (Fig 5).While on day 12 and 18 granulation tissue collected was not sufficient for doing all the biochemical tests because of better wound contraction

Tensile strength

A significant increase in the skin tensile strength of the leaf and flower extract treated group on the post wounding day (Table 2). Tensile strength is the maximum stress a material can withstand under testing. From the data obtained, it was observed that flower extract treated group has the highest tensile strength when compared with leaf extract and control groups. When we consider the maximum percentage strain it clearly reveals that flower extract treated group has 80% strain than control which has only 25.4 % strain. Overall the results prove that all healed tissues treated with flower extract had higher tensile strength than the control and leaf extract treated groups.

Histological Examinations

Histological examination reveals that there was a higher expression of macrophages and mast cells in the treated groups than in the control group (Fig 6). The sections of granulation tissue of extract treated animals showed the sign of tissue repair with increased collagen formation and less macrophage. Whereas, in the control animals the healing activity was comparatively lesser with moderate collagenation and retention of the macrophages. There was a substantial increase in fibroblast and collagen density in the flower extract treated group than the leaf extract treated and control groups. It should be mentioned that there is a moderate amount of edema in the control group. Interestingly, flower extract treated group showed a clear epidermal layer containing epithelial cells (pinkish layer) interspersed with cells indicating collagen synthesis in the regenerating dermal tissue.

DISCUSSION

The screening of plant extracts has been of great interest to scientist for the discovery of new drugs effective in the treatment of several diseases. Indian flora has one of the most extensive floras in the world with more than 9000 plant species. A number of reports concerning the antibacterial, anti-inflammatory and wound healing activity of plant extracts of Indian medicinal plants have appeared in the literature, but the vast majority has yet to be investigated.

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 depends upon the reparative abilities of the tissue, type and extent of the damage and general state of the
health of the tissue. Granulation, collagen maturation and scar formation are some of the many phases of wound healing, which run concurrently, but independent of each other (25). The granulation tissue of the wound is primarily composed of fibroblast, collagen, edema, and small new blood vessels. The undifferentiated mesenchymal cells of the wound margin modulate themselves into fibroblast, which start migrating into the wound gap along with the fibrin strands. The collagen is the major component of extra cellular tissue, which gives support and strength and is composed of amino acid (Hydroxyproline).

Wound contraction is thought to be mediated by specialized fibroblasts found within granulation tissue (26). The ability of fibroblasts to contract collagen gel is considered to be a specific function, which exists *in vivo*. Increased wound contraction in the treated group may be due to the formation of fibroblasts; increased fibroblast production in turn activates the production of collagen (27, 28).

In the present study, the total protein content of the granulation tissue of the wound treated with leaf and flower extract was found to be higher

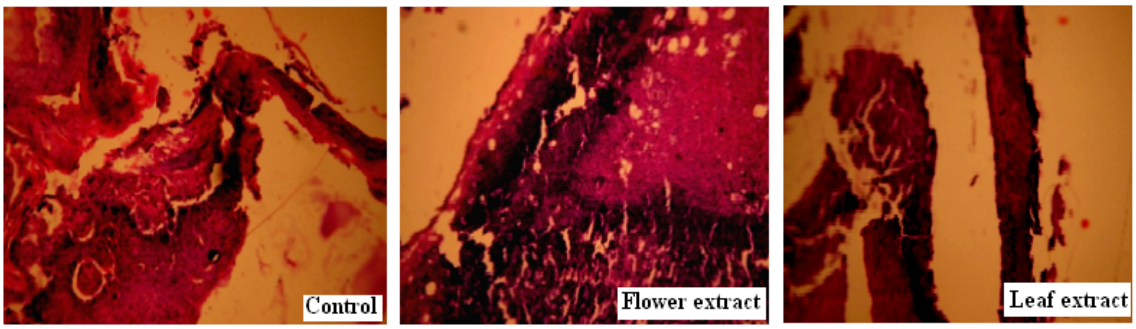

Fig 6. Histopathological report

than control. The fact that the extract treated group had a higher protein content than the control group may be due to either cellular infiltration or increase in collagen synthesis. Higher the protein content implies higher metabolic rate for wound healing. In wound healing, protein metabolism is fundamental for the repair of collagen, a tissue which is dependent on the synthesis of a large quantity of special protein. The hexosamine content of granulation tissue of the wound treated with flower extract was found to be higher than control and leaf extract treated group. By correlating hexosamine content it can be judged how fast the wound heals. It is important to note that hexosamine content will increase during wound healing process and decreases when maturation and remodeling phase is attained. The hydroxy proline and total uronic acid content of the granulation tissue of the wound treated with *extract* was found to be higher than control. Hydroxyproline is a major component of the protein collagen. They permit the sharp twisting of the collagen helix and provide stability to the triple-helical structure of collagen by forming hydrogen bonds. For this reason, hydroxyproline content has been used an indicator to determine collagen amount. Wound healing involves interactions of multiple cell types with various cytokines, growth factors, their mediators, and the extracellular protein fibronectin, laminin, tenascin, and collagen (29). The increased hydroxyproline content agrees with the increase in protein content, which is predominantly due to enhanced collagen synthesis in the treated group. Decrease in uronic acid content was observed in the treated group. The decrease in uronic acid is attributed to an increase in collagen synthesis. This was further supported by Cohen and Haynes, who found that an increase in uronic acid will lead to a decrease in collagen synthesis (30, 31)

Union of the two sides of a wound is believed to be made by the deposition of collagen fibers in a matrix of granulation tissue. Studies of the granulation layer, however, suggested that the changes originated in cells of the surrounding tissue. This concept is supported by several works which showed that new fibroblasts develop in the tissue around wounds (32, 33).

A significant increase in the skin tensile strength of the leaf and flower extract treated group on the post wounding day. Histological examination reveals that there was a high migration of inflammatory cells toward the wound environment in treated groups. High migration of inflammatory cells expresses a wide variety of cytokines and functions to aid in tissue repair in treated groups (34). An increase in the expression of cytokines activates fibroblasts toward the wound environments. The increase in fibroblast and collagen expression in wound sites by histological examination correlates with the above results, and it is supported by an increase in hydroxyproline content in extract treated groups. Migration of inflammatory cells, a high expression of fibroblasts and collagen, and an increase in wound contraction reveal that extract treated groups follows the normal wound-healing cascade of inflammation, proliferation, and scar formation. Many plant extracts and medicinal herbs have shown potent antioxidant activity. Tannins the main components of many plant extracts, act as free radical scavengers (35, 36).

It has been evident that phytoconstituents such as catechins can significantly improve the quality of wound healing and scar formation (37), flavonoids because of their antioxidant property accelerates the wound healing process and could be a potential therapeutic tool in the treatment of SMC-rich vascular lesions (38, 39), triterpenoids (40), flavonoids, saponins (41) etc., are known to promote the wound healing process due to their antioxidant and antimicrobial activities. Earlier reports reveal that the plant contains many active constituents such as glucosides, terpenoids, flavonoids etc., which are known for their antioxidant property, the present study reveals that LC possess significant wound healing promoting activity which may be attributed to the active constituents present. Hence the present study provides a scientific base for the ethnomedicinal property of LC.

Conclusion:

This finding thus, justifies its use in folkloric medicine for wound healing. At this stage, it is difficult to say which component(s) of the extracts are responsible for wound healing activity. However, further phytochemical studies are needed to isolate the active compound(s) responsible for these pharmacological activities.

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