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Wound healing potential of *Launaea pinnatifida C*ass leaf juice in rats

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ABSTRACT

Launaea pinnatifida is a procumbent herb native to the coastal area and found throughout India and many other countries. It is utilized as a dietary plant in the preparation of various dishes in the Maldives. Traditionally it is used for the treatment of skin injuries and is reported to contain flavonoids, steroids, glycosides, tannins, saponins etc. As no scientific work has been found to be published related to wound healing potential of the *L. pinnatifida* plant, the present study investigated the effects of *dried leaf juice of L. pinnatifida* on wound healing property in rats using various wound models. In result analysis, the wound contraction percentage was found to be increased and the scar area and epithelization time were found to be decreased when 3 % ointment *L. pinnatifida leaf juice* was applied topically. Similarly, with incision and dead space wound models, wound breaking strength and hydroxyproline levels were found to be increased. So, *Launaea pinnatifida* leaf juice showed significant wound healing activity in all wound models, which supports its claim for being used traditionally in skin diseases.

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INTRODUCTION

Wound healing is a dynamic process where skin/tissues get repaired following an injury. It is a response in which an inflammatory phase followed by the synthesis of collagen and other extracellular matrix ultimately lead to the scars. The process of wound repair is a complex process involving various phases such as Hemostasis, inflammation and proliferation (Bodeker *et al.*, 1996).

A large no. of drugs of plant and animal origin have been described in Ayurveda and other systems of medicine for the treatment of skin diseases and many more are traditionally being used by the local tribes for healing wounds. Most of these drugs are obtained from the plants and have been evaluated for their wound healing potential scientifically, but still, there are many more drugs from natural sources which need to be explored (Biswas and Mukherjee, 2003).

*Launaea pinnatifida (*Syn. *L. sarmentosa)* belongs to family Asteraceae. It is commonly known as Sagar pathari and is a glabrous, procumbent herb with slender stems and radical, oblong, toothed leaves. The plant bears solitary dark yellow flower heads. In the coastal region, it is found to be a useful sand binder (Nadkarni, 1982). It has been shown to possess tonic, diuretic, galactogogue and soporific properties. In Goa, it is traditionally used as a substitute for Dandelions (Kiritkar and Basu, 2001). Plant Juice is used in the treatment of skin diseases and

rheumatic affections (Tetali *et al.*, 2009). Two components, taraxasteryl acetate from roots and taraxasterol from leaves, have been reported to be isolated (Rastogi and Mehrotra, 1999). The present study was aimed to evaluate the *Launaea pinnatifida* leaf juice potential as wound healer in rats by various wound healing models.

MATERIALS AND METHODS

Launaea pinnatifida plants were collected from the Candolim beach, North Goa, India. The plant was identified and authenticated by the Department of Botany, RTM Nagpur University, Nagpur.

Preparation of juice

Fresh Leaves of *L.pinnatifida* were separated from the stems and thoroughly cleaned with distilled water. They were then cut into small pieces and the juice was obtained by grinding with little distilled water in an electrical grinder. The resulting slurry was filtered and evaporated to dryness. The yield was found to be 15 %.

Ointment of leaf juice (3 % w/v) of *L. pinnatifida* was formulated using simple ointment IP as a base. In excision model, 0.5 g of each of ointment was applied daily to the animals. In incision and dead space wound models animals, an aqueous juice solution was administered in the dose of 250 mg/kg and 500 mg/kg.

Selection of Animals

Healthy Sprague Dawley rats (150-250g) were selected for wound healing potential studies. They were kept under standard conditions of light and Temperature, fed with commercial feed and water. The protocol was approved with Institutional Animal Ethical Committee for experimental clearance.

Acute toxicity testing

Acute toxicity studies of Launaea *pinnatifida* leaf juice was carried out according to Lorke (1983), with few modifications. Rats (n = 6) were fasted for 16 h before the oral administration of *L. pinnatifida* leaf juice in the dose of 500, 1000, 2000, 3000, 4000 and 5000 mg/kg. Additional group of rats was used as control and it received physiological saline orally. All the experimental animals had free access to food and water over 5 days of observation period and they were examined for any acute toxicity symptoms and any deaths.

In vivo wound healing activity Excision wound model

The animals were anaesthetized using Ketamine HCL (120 mg/kg, IP) and hairs were removed from their dorsal thoracic region. A full thickness circular excision wound of size about 500 mm^2 was made on each rat. Separate group (n = 6) of rats were treated topically with an ointment containing leaf juice of *Launaea pinnatifida* (3 % w/v) (500mg/rat per day) or simple ointment base or Povidone iodine ointment (500 mg/rat per day) every day for about 16 days. On the day of wounding and on day 4, 8, 12, 16, wound contraction was measured by tracing wounds on graph paper until complete healing occurred. Wound contraction rate and the epithelization period were calculated as the number of days required for shading off of the complete dead tissue (Morton and Malone, 1972).

Incision Wound Model

On either side of the vertebral column of rats, two incisions of about 6 cm long were made through the full thickness of the skin and the wounds were bunged by means of 1 cm apart interrupted sutures. A separate group of rats were daily administered with saline (10 ml/kg, oral) or leaf juice (250 and 500 mg/kg, oral) or applied topically with Povidone iodine ointment (500 mg/rat per day), (Ehrlich and Hunt, 1969).

The sutures were removed on the 7th day of wounding and on 10^{th} day, the wound breaking strength was checked using water flow technique as described in the literature (Lee, 1968).

Dead Space Wound

Two polypropylene tubes of size (0.5×2.5 cm^2 each) were implanted to create a wound on a separate group of animals on the dorsal surface of their either side of the lumbar region (Patil PA,1984). From 0 - 9 day of wounding, the animals received saline (10 ml/kg, oral) or leaf juice (250 and 500 mg/kg, oral) or applied topically with Povidone iodine ointment (500 mg/rat per day).The tissues from each implanted tube were dissected out on day 10, along with the tube and the content of hydroxyproline and granuloma breaking strength were estimated (Woessner, 1961).

Statistical Analysis

By using one way analysis of variance (ANOVA) with post hoc Dunnett test or Neumann-Keultest, the results were analyzed. P value less than 0.05 was considered to be statistically significant.

Acute toxicity studies

The juice of the leaves of plant *Launaea pinnatifida* was found to be safe (5000 mg/kg) by the oral route. The rats were found to be well tolerated after 24 h and no symptoms of toxicity and mortality were observed during the 14 days of post administration observation period.

RESULTS

Excision wound method

The ointment of dried leaf juice of *Launaea pinnatifida*, on topical application, increased the percentage of wound contraction, as shown in Figure 1. The progression of wound healing was accelerated by 12^{th} day, i.e. (70.06± 3.28, P < 0.05) compared with control (60.96 ± 2.29) and the time of epithelization was also reduced from 21.83 ± 1.14 to 18.50 ± 0.76 days [F (2, 17) = 6.28, P< 0.05] as compared with simple ointment treated animals (Figure 2A). Moreover, *Launaea pinnatifida* leaf juice ointment treated rats also exhibited significantly reduced scar area from 51.73±3.95 to 38.82±2.29mm^{2}when compared with control animals [F(2,17)=7.77, P < 0.05] (Figure 2B). < 0.05, ** P < 0.01 when compared against the animals which were applied topically with simple ointment base (One way ANOVA post hoc Dunnett test).

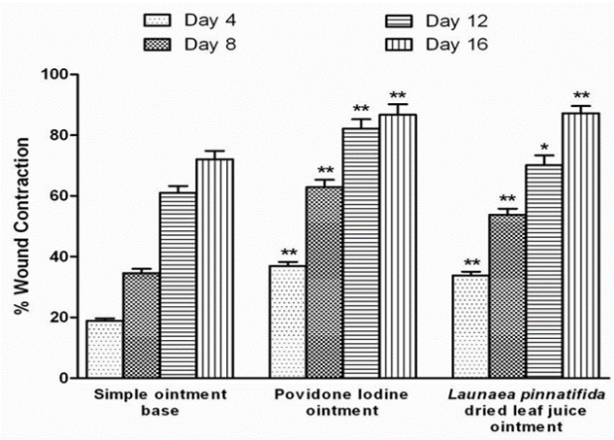

Figure 1: Each column represents the % Mean ± SEM (n = 6)wound contraction

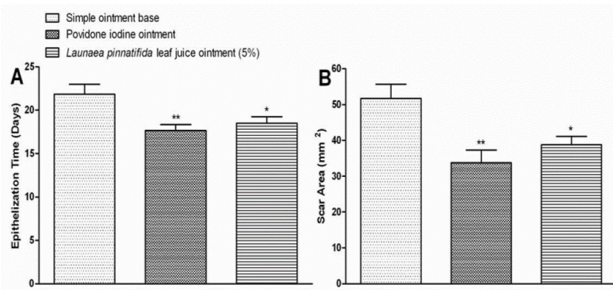

Figure 2: Each column represents the % Mean ± SEM (n = 6)wound contraction

Incision wound method

As depicted in Figure 3, the breaking strength was significantly (P < 0.01) found to be increased in *Launaea pinnatifida* leaf juice (500 mg/kg/animal) treated groups (386.9 ± 29.52) as compared to saline treated animals (265.6 ± 25.33) [F (3, 23) =

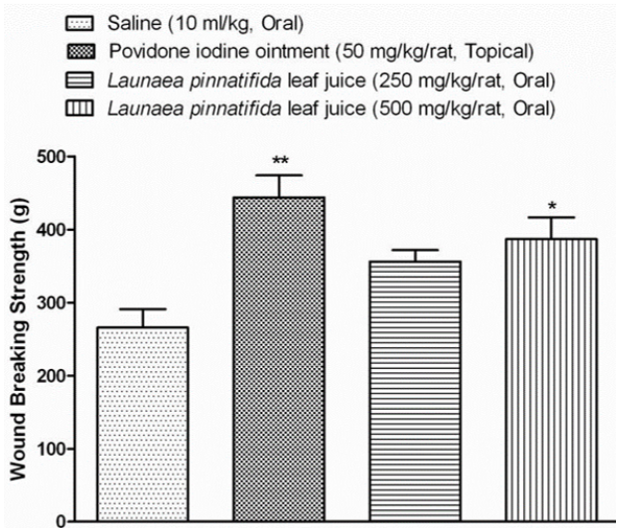

Figure 3: Each column represents the Mean ± SEM (n = 6)

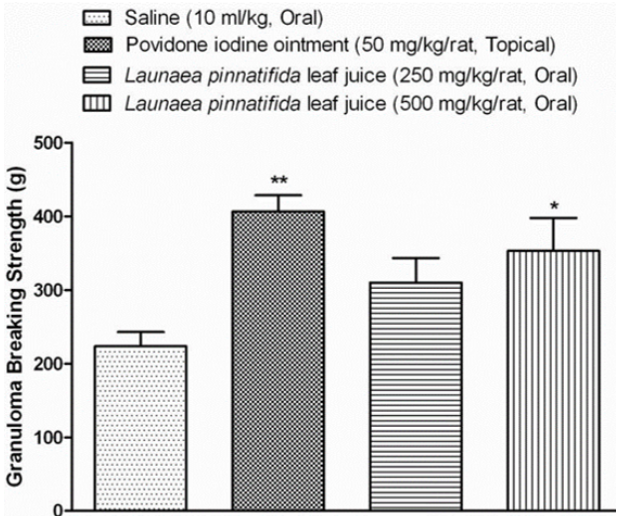

Figure 4: Each column represents the Mean ± SEM (n = 6)

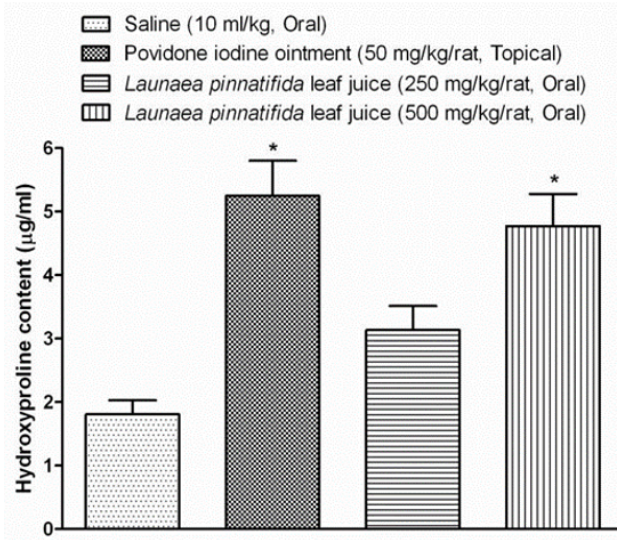

Figure 5: Each column represents the Mean ± SEM (n = 6)

8.26, P < 0.001]. * p< 0.01, ** P < 0.001 compared against the saline treated control animals (One way ANOVA post hoc Dunnett test). However, the increment in breaking strength from *Launaea Pinnatifida* leaf juice (250 mg/kg/animal) treated rat was statistically insignificant. These results were comparable to the wound strength determined in rats treated with povidone iodine ointment.

Dead space wound method

As shown in Figure 4, there was a significant increase in granuloma breaking strength in *Launaea pinnatifida* dried leaf juice (500 mg/kg/animal) treated groups (352.8 ± 44.88) when compared with saline treated animals (223.5 ± 19.40) [F (3, 23) = 5.99, P < 0.01]. * p< 0.05, **< 0.01 compared against the saline treated control animals (One way ANOVA post hoc Dunnett test). In addition, there was also considerable increase in hydroxyproline content in *Launaea pinnatifida* leaf juice (500 mg/kg/animal) treated rats [F (3, 23) = 13.13, P < 0.001] As Show in (Figure 5), * p< 0.05, **P< 0.01 compared against the saline treated control animals (One way ANOVA post hoc Dunnett test).

Topical application of povidone iodine ointment also showed an increase in the granuloma breaking strength (P < 0.01) and also hydroxyproline content (P < 0.001) as compared to control animals. However, administration of *Launaea pinnatifida* leaf juice (250 mg/kg/animal) failed to show any significant increase in both the parameters as compared to saline treatment.

DISCUSSION

The wound healing process involves various phases such as granulation,collagenization, collagen maturation etc., which occur independently at the same time. To represent the various phases of wound healing collectively, the use of any single model is insufficient, so three different animal models were used to evaluate the effect of *Launaea pinnatifida* leaf juice on various phases.

In the present study, *Launaea pinnatifida* leaf juice showed considerable wound healing potential in all the three animal models. In excision model, it showed a significant increase in wounds contraction through enhanced epithelization, which may be on account of its effect on increased collagen synthesis.

And in the incision model, there was increased breaking strength of the incision wounds, which may be due to the increase in collagen concentration and stabilization of the fibers (Udupa *et al.*, 1995). Similarly, in dead space wound model, there was a significant increase in both granuloma tissue breaking strength and hydroxyproline content.

The increased amount of hydroxyproline in test groups underlines increased collagen content, as hydroxyproline is the direct estimate of collagen synthesis (Madden and Peacock, 1968).

Preliminary phytochemical screening of juice showed various phyto-constituents such as flavonoids, tannins, saponins, sterols etc. which are reported to be involved in the wound healing process due to their antimicrobial, astringent and antioxidant characteristics.

The bioactivity of flavonoids is strongly correlated with their chemical structure and action mechanisms, mostly inhibitory activity on enzymatic systems involved in cellular activations (Ielpo *et al.*, 2000; Devipriya and Shyamaladevi, 1999).

Moreover, *Launaea pinnatifida* has been shown to possess the remarkable free radical scavenging activity (Nagalpar and Paramjyoti, 2010).

In the skin, biological activities are due to its interaction with various binding proteins and in addition, molecular oxygen plays a key role in the pathogenesis and therapy of chronic wounds.

Overproduction of reactive oxygen species (ROS) results in oxidative stress which results in cytotoxicity and delayed wound healing. Therefore, elimination of ROS could be an important strategy in the healing of chronic wounds (Dissemond *et al.*, 2002).

The role of antioxidants in hastening the process of wound healing by destroying the free radicals (Halliwell *et al.*, 1988) and by increasing wound breaking strength was experimentally proved (Michel, 1990).

CONCLUSIONS

The present study about the effect of *Launaea pinnatifida* leaf juice on wound healing,showed better results with a dose of 500 mg/kg body weight. As *Launaea pinnatifida* grows wildly in coastal areas, so it could be used a cheap and easily available therapeutic agent for the management of wound and other skin related conditions.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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