Chapter 5
Wound Healing Activity
Introduction

The wound may be defined as a loss or breaking of cellular and anatomic or functional continuity of living tissues (Nalwaya et al., 2009). The skin serves as a protective barrier against the outside world, any break in it must be rapidly and efficiently mended (Martin, 1997). Healing of wounds is an important biological process involving tissue repairs and regeneration (Esimone et al., 2009). It is a complex and dynamic process of restoring cellular structures and tissue layers (Mercandetti and Cohen, 2007). Proper healing of wound is essential for the restoration of disrupted anatomical continuity and functional status of the skin (Annan and Dickson, 2008).

The concept of developing drugs from plants, which are used in indigenous medicine system is much older. While in some cases direct links between a local and biomedical use exists, in other cases, the relationship is much more complex (Heinrich and Gibbons, 2001). Wounds, particularly, chronic wounds are major concerns for the patient and clinician. Chronic wounds affect a large number of patients and seriously reduce their quality of life. There are very few Indian studies on the epidemiology of chronic wounds (Gupta et al., 2004). Current estimates indicate that, nearly 6 million people suffer from chronic wounds worldwide (Sasidharan et al., 2010). Research on wound healing agents is one of the developing areas in modern biomedical sciences. Many traditional practitioners across the world, particularly, in countries like India and China, with age old traditional practices have valuable information of many lesser-known and wild plants used by the traditional healers for treating wounds and burns. Several
drugs of natural products, such as plants, animal and mineral origin are described in the traditional texts of Indian systems of medicine like Ayurveda, for their healing properties under the term 'Vranaropaka' (Kumar et al., 2007).

According to WHO, more than 80% of the world’s population, still depends upon the traditional medicines for the treatment of various diseases (Patel et al., 2009). In folk medicine, some medicinal plants have been working for healing wound. Some of these plants either possess pro-wound healing activities or exhibit antimicrobial and other related properties, that are beneficial in overall wound care (Esimone et al., 2009).

Recently, the traditional use of plants for wound healing has received attention by the scientific community. About one-third of all traditional medicines used for the treatment of wounds and skin disorders, which is against only 1-3% of modern drugs (Ghasemi et al., 2010). In the view of increased wide spectrum of medicinal usages, the present day requires new biologically active ointments which exhibit wound healing activity as local applications. Wound healing studies are mainly aim to detect various means and factors influencing healing process (Sachin et al., 2009).

Review of Literature

Wound is defined simply as the disruption of the cellular and anatomic continuity of a tissue (Bennet, 1988). Wound may be caused 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. Therefore, the aim of treating a wound is to either shorten the time required for healing or to minimize the undesired
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Consequences. Attention should be directed towards discovering an agent, which will accelerate wound healing either when it is progressing normally, or when it is suppressed by various agents like corticosteroids, anti-neoplastics, or non-steroidal anti-inflammatory agents (Raina et al., 2008).

The literature collected pertaining to the study of wound healing activities of *Epiprinus mallotiformis* has interpreted meaning fully in this review part, which are as follows:

In 1998, Kumar et al. evaluated that, topical formulations of aqueous extract of *Centella asiatica* on open wounds in rats. Aqueous extract of *Centella asiatica* was treated to wound when applied topically, thrice daily for 24 days on the open wounds in rats increased cellular proliferation and collagen synthesis at the wound site, as evidenced by increase in collagen content and tensile strength. The treated wounds epithelialised faster and the rate of wound contraction was higher as compared to control wounds.

Anonymous (2000) evaluated the methanolic extract of rhizomes of *Nelumbo nucifera* in the formulation of ointment to effective in different types of wound model in rats. The effects were studied on excision wound model, incision wound model and dead space wound model. The ointment responded significantly in all the wound models. The extract ointment shows the significant effect in respect with wound contracting activity, wound closer time, tensile strength, regeneration of tissue at the wound site.

In 2001, Bairy et al. studied on *Gingko biloba*. They found that *Gingko biloba* extract has significant wound healing activity against both dead space and excision wound models in male rats. 50 mg/kg of dose has significantly promoted the breaking
strength and hydroxyproline content of granulation issue in dead space wounds and in case of excision wound model, it is found to shorten the epithelization period.

Suguna et al. (2002) reported on the effects of topical administration of an alcohol extract of the leaves of an evergreen plant, *Terminalia chebula*, on the healing of rat dermal wounds, *in vivo*. *T. chebula* treated wounds healed much faster as indicated by improved rates of contraction and a decreased period of epithelialization. Biochemical studies revealed a significant increase in total protein, DNA and collagen contents in the granulation tissues of treated wounds.

Priya et al. (2002) have been investigated the alcohol extract of the *Datura alba* leaves for the evaluation of its healing efficiency on burn wound models in rats. A 10% (w/w) alcoholic extract was topically applied on thermal wounds. Complete wound closure was observed within 12 days in treated rats. Wound closure time, tissue regeneration at the wound site and histopathological characteristics were significant in treated rats. Collagen, hexosamine and gelatinase expressions were also well correlative with the healing pattern observed. In the same year 2002, Malviya et al. have investigated the aqueous extract of the roots of *Radix paeoniae* was screened for wound healing by excision, incision and dead space wound models on Wistar rats, reduction in the wound breaking strength when compared to control group in incision type of wound model. The results obtained indicated that *Radix paeoniae* root extract accelerates the wound healing process by decreasing the surface area of the wound and increasing the tensile strength. The histological examination of the granulation tissue of treated group showed increased cross-linking of collagen fibers and absence of monocytes.

Reddy et al. (2002) studied on the ethanolic extracts of *Heliotropium indicum*, *Plumbago zeylanicum* and *Acalypha indica* for their wound healing activity in rats.
Wound healing activity was studied using excision and incision wound models in rats following topical application. *H. indicum* possesses better wound healing activity than *P. zeylanicum* and *A. indica*. Tensile strength results indicate better activity of *H. indicum* on remodeling phase of wound healing.

Shirwaikar *et al.* (2003) have investigated the leaves ethanol extract of *Aristolochia bracteolate* for wound healing activity by different models such as incision, excision and dead-space in rats, in ethanol extract 800 mg/kg concentration shows the significant results was found for wound healing activity.

Mathew *et al.* (2004) have investigated for wound healing activity of *Gentiana lutea* Rhizome, extracts exhibited significant wound healing activity at 300 and 500 mg/kg, p.o., in excision, resutured incision and dead space wound models.

Manjunatha *et al.* (2005) have studied on evaluation of wound healing potency of *Vernonia orborea*. The methanol extracts of plant was applied for three different wound healing models such as excision, incision and dead space. Aqueous and methanolic extracts promoted the wound healing activity significantly in all the wound models. The aqueous extract of *Carica papaya* leaves were investigated for evaluation of wound healing potential in rats (Mahmood *et al.*., 2005).

Singh *et al.* (2005) investigated that, the effect of aqueous ethanol extracts and the isolated compound deoxylephantopin from *Elephantopus scaber*. The wound healing was studied carried out by excision, incision, and dead space wound models in rats. The wound-healing activity was assessed by the rate of wound contraction, period of epithelialization, skin-breaking strength, weight of the granulation tissue, and collagen content. The ethanol extract and the isolated constituent deoxylephantopin of *E. scaber* promoted wound-healing activity in all the three wound models.
In 2006, Jain et al. reported on the aqueous extract of *Desmodium gangeticum* and evaluated for its wound healing properties. 10% (w/w) concentration showed the significant result of tensile strength and epithelization. One of the medicinal liver wort, *Plagiochasma appendiculatum* extract has potent wound healing capacity as evident from the wound contraction and increased tensile strength (Singh et al., 2006). Ethanol extract of *Aloe vera* leaf was evaluated for excision wound, the result shows the increased in the collagen and extracellular matrix in the ethanol treated animals when compare with the standard (Subramanian et al., 2006).

Udupa et al. (2005) have investigated the ethanolic extract of leaves of *Ocimum sanctum* for normal wound healing and dexamethasone depressed healing using incision, excision and dead space wound models in albino rats. The extract of *O. sanctum* significantly increased the wound breaking strength in incision wound model. The extract treated wounds were found to epithelialize faster and the rate of wound contraction was significantly increased as compared to control wounds, significant increase in wet and dry granulation tissue weight, granulation tissue breaking strength and hydroxyproline content in dead space wound model. Trombetta et al., 2006 reported that the extracts of *Opuntia ficus-indica* cladodes are used in folk medicine for their antiulcer and wound-healing activities.

In 2007, Khalil et al. have investigated the wound healing effect of some Jordanian traditional medicinal plants formulated in Pluronic F127 using mice. Aqueous extracts of *Inula viscosa, Ajuga chia, Rubia taenifolia* and *Parieteria diffusa* and the oil of *Laurus nobilis*, were examined for their wound healing activity out of them the best wound healing activity was observed with the extract of *Inula viscosa*, followed by *Parieteria diffusa, Laurus nobilis, Ajuga chia* and the least active extract was that of *Rubia taenifolia*.
Kumaraswamy et al. (2007) worked on wound healing activity of embelin isolated from the ethanol extract of leaves of *Embelia ribes*. Ethanol extract of the leaves of *Embelia ribes* and its isolated quinone compound embelin were screened for wound healing activity by excision, incision and dead space wound models on *Swiss Albino* Rats. Significant wound healing activity was observed in both ethanol crude extract (30 mg/ml) and the constituent treated groups.

Puratchikody et al. (2007) have been investigated the wound healing activity of leaf extract of *Memecylon umbellatum*. The incision, excision model shows the significant activity when compare to control group. Pattanayak et al. (2008) investigated the ethanolic extract of aerial parts of *Dendrophthoe falcata* for the evaluation of its healing efficiency on excision and incision wound models in rats. The results showed that *Dendrophthoe falcata* extract has potent wound healing capacity as evident from the wound contraction and increased tensile strength.

Edwin et al. (2008) studied on *in vivo* wound-healing efficacy and antioxidant activity of *Achyranthes aspera* in experimental burns. The ethanol and aqueous extracts of leaves of *Achyranthes aspera* were prepared and its wound healing activity was evaluated. The wound healing activity was studied using two wound models, excision wound model and incision wound model, 5% ointment of *A. aspera* showed significant. Wound healing the extracts responded significantly in both the wound models tested.

Nayak et al. (2008) have been investigated the wound activity of *Lantana camara*. The ethanol extract of *L. camara* increased the rate of wound contraction by 87% in burn wound when compared to controls (82%).

Karodi et al. (2009) studied on cuts, wounds and burns. The alcoholic extract of *Rubia cordifolia* was investigated for the evaluation of its healing efficiency on excision
wound model in mice, *R. cordifolia* and found to be effective in the functional recovery of the healing of wounds and also in histopathological alterations.

Akkol *et al.* (2009) investigated that the root extract of *Arnebia densiflora* for its wound healing activity by using hexane, chloroform, ethyl acetate and methanol solvent extracts. Incision and excision models was employed to study the wound healing activity. Significant wound healing activity was observed with the ointment formulation prepared by using hexane extract. The results of histopathological examination supported the outcome of both incision and excision wound models.

Dnyaneshwar *et al.* (2009) studied on evaluation of wound healing activity of root of *Mimosa pudica*. Wound healing activity was studied in three types of model in rats viz. excision, incision and estimation of biochemical parameter. In case of the excision wound model wound contraction and period of epithelization was studied while in incision wound model was evaluated by determining tensile strength and hydroxyproline content in the scab. Treatment of wound with ointment containing 2% (w/w) the methanolic and 2% (w/w) the total aqueous extract exhibited significant wound healing activity.

Deshmukh *et al.* (2009) investigated the effects of *Calotropis gigantea* root bark on wound healing activity in rats by excision, incision and dead space wound healing models in rats. Topical application of *Calotropis gigantea* in excision wound model increased the percentage of wound contraction. Scar area and epithelization time were decreased. In incision wound and dead space wound breaking strength of wounds and hydroxyproline was increased. Leaves of *Memecylon edule* was extracted by hexane, ethyl acetate, methanol evaluated for wound healing activity ethyl acetate extract shows the significant activity (Nualkaew *et al.*, 2009).
In 2010, Bharathi et al. studied on wound healing activity of *Stereospermum colais* leaves by excision model, leaves were extracted from chloroform, ethanol and aqueous extracts and they were screened. Chloroform and ethanol extract showed significant activity when compared with control.

Sasidharan et al. (2010) worked on wound healing potential of *Elaeis guineensis* leaves in an infected albino rat model. The wound healing activity of leaves of *E. guineensis* was methanolic extract in yellow soft paraffin in concentration of 10% (w/w). The results show that the *E. guineensis* extract has potent wound healing capacity, as evident from better wound closure, improved tissue regeneration at the wound site and supporting histopathological parameters pertaining to wound healing.

Akilandeswari et al. (2010) investigated the wound healing activity of *Sida acuta*. The Effects of topical administration of methanolic extract of *Sida acuta* ointment was studied respectively on two types of wound models in rats, (i) the excision and (ii) the incision wound model. The ointment of the methanol extract of *Sida acuta* produced significant response in both of the wound types tested. In the excision model the extract treated wounds were found to epithelialise faster and the rate of wound contraction was higher, as compared to control wounds. The extract facilitates the healing process as evidenced by increase in the tensile strength in the incision model.

Ahmed et al. (2012) reported on *Phyllanthus niruri* leaf extract. Grossly, wounds treated with placebo containing 5%, 10% *P. niruri* extract or Intrasite gel significantly accelerated the rate of wound healing compared to wounds treated with sterile deionized water. Wounds dressed with placebo containing 5%, 10% *P. niruri* extracts or Intrasite gel showed markedly less scar width at the wound enclosure with large amounts of
fibroblasts proliferation, more mature and densely packed collagen and angiogenesis compared to wounds dressed with sterile deionized water or blank placebo.

Solanki and Jain (2012) investigated on the root and seed extract *Clitoria tenatea* and evaluated for wound healing activity by excision, incision and dead space wound model. Both the extract significantly improves the wound healing in all the three models, the finding suggested that *C. tenatea* affect the all three phases-inflammation, proliferation and remolding phase. Deepti *et al.* (2012) have been evaluated wound healing properties of *Coccinia grandis*. The chloroform extract shows the significant epithelization in all the wound healing models.

Garg *et al.* (2012) revealed that whole plant of *Viscum articulum* for wound healing activity in albino rats. The extract of whole plant of *V. articulatum* Brm was evaluated for its wound healing activity in albino rats using excision, incision and dead space wound. The reference standard was treated with Soframycin (1%) ointment. The extract treated animals exhibit $63\pm0.7032 \text{ mm}^2$ reduction in wound area when compared to controls which was $71.67\pm0.4944 \text{ mm}^2$. The extract treated wounds are found to epithelize faster as compared to controls. Significant $0.001$ increase in granuloma breaking strength $(420.801\pm3.97)$ and dry granulation tissue $40.267\pm2.513$ was observed.

Talekar *et al.* (2012) studied on evaluation of wound healing potential of aqueous and ethanolic extracts of *Tridax procumbens* by using both excision and incision wound model. In incision wounds, tensile strength of the wound in the drug treated animals was increased much more significantly as compared with control group animals. In excision wound model the rate of wound contraction was assessed as healing parameter at every 3 biochemical tissue markers like Hydroxyproline, Collagen and Hexosamine were
determined from excised tissue and they were significantly increased in plant extract treated groups.

This study aimed at investigating the wound healing activity of the leaf and bark extracts and isolated pure compound from this ethnic Indian medicinal plant *E. mallotiformis*, in order to validate or otherwise prove the claims of the herbalists, who use it as a wound healing remedy. This study will also hopefully expose new frontiers by improving the current applications of the plant extracts.

**Materials and Methods**

**Excision Wound**

The excision wound model is employed to assess the potency of drug to promote the wound healing in ‘Trauma’ types of wound, which will be assesses by the rate of wound contraction and number of days required for complete epithelialization of the wound area. The rats were inflicted with excision and incision wound as described by Morton and Malone (1972) and Ehrlich and Hunt (1969) respectively. The wound were traced on mm2 graph paper on the days of 4th, 8th, 12th and 16th and thereafter on alternate days until healing was complete. The number of days required for falling of the scar without any residual of the raw wound gave the period of epithelialization.

Albino rats of Wistar strain weighing 150-200 g were procured from the central animal house, National College of Pharmacy, Shimoga, Karnataka, India. Albino rats were divided into 11 groups of six rats in each group. Group I considered as the control, Group II served as reference standard and treated with 0.2% w/w Nitrofurazone ointment, group III, IV and V were administered ointment based petroleum jelly with the crude
methanolic leaf extract of *E. mallotiformis* at the dose of 100, 200 and 300 mg/kg respectively group VI, VII and VIII animal treated ointment based petroleum jelly with the methanolic bark extract of *E. mallotiformis* at the dose of 100, 200 and 300 mg/kg body weight and Group IX, X and XI animal treated ointment based petroleum jelly with the isolated pure compound, 13-labdene-2,3,8,15-tetrol from leaves of *E. mallotiformis* at the dose of 100, 200 and 300 mg/kg body weight respectively.

**Incision Wound**

In incision wound model, the tensile strength is the resistance to breaking under tension. It indicates, how much the repaired tissue resists to breaking under tension and may indicate in part the quality of repaired tissue. This was observed by following the method of Ehrlich and Hunt (1969). The newly formed tissue including scar was excised and tensile strength was measured, with the help of tensiometer, which is based on method of Lee (1968).

Albino rats of Wistar strain weighing 150-200 g were procured from the central animal house, National College of Pharmacy, Shimoga, Karnataka, India. Albino rats were divided into 11 groups of six rats in each group viz., Group I considered as the control, Group II served as reference standard and treated with 0.2% w/w Nitrofurazone ointment, group III, IV and V were administered orally with the crude methanolic leaf extract of *E. mallotiformis*, at the dose of 100, 200 and 300 mg/kg respectively group VI, VII and VIII animal treated orally, with the methanolic bark extract at the dose of 100, 200 and 300 mg/kg body weight and Group IX, X and XI animal treated orally, with the isolated pure compound, 13-labdene-2,3,8,15-tetrol from leaves of *E. mallotiformis* at the dose of 100, 200 and 300 mg/kg body weight respectively.
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Wound Healing Activity

Dead Space Wound

Dead space wounds were created by subcutaneous implantation of sterilized cylindrical grass piths (2.5 cm x 0.3 cm), one on either side on the dorsal paravertebral surface of the rats under light ether anesthesia (Patil and Kulakarni, 1984). The granulation tissue formed around the grass pith was excised on 10th post wounding day and breaking strength was measured. Simultaneously the granulation tissues were harvested and subjected to histological study.

Albino rats of Wistar strain weighing 150-200 g were procured from the central animal house, National College of Pharmacy, Shimoga, Karnataka, India. Albino rats were divided into 11 groups of six rats in each group. Group I considered as the control, Group II served as reference standard and treated with 0.2% w/w Nitrofurazone ointment, group III, IV and V were administered orally with the crude methanolic leaf extract of *E. mallotiformis* at the dose of 100, 200 and 300 mg/kg respectively group VI, VII and VIII animal treated orally with the methanolic bark extract at the dose of 100, 200 and 300 mg/kg body weight and Group IX, X and XI animal treated orally with the isolated pure compound, 13-labdene-2,3,8,15-tetrol from leaves of *E. mallotiformis* at the dose of 100, 200 and 300 mg/kg body weight respectively.

Histology

For the histological studies, wound tissue specimens from test animals taken as control, experimental and standard groups were taken after complete healing of dead space wound. After usual processing, 6 mm thick sections were cut and stained with hamatoxylin and eosin (McManus and Mowry, 1965). Later sections were qualitatively assessed under the light microscope and observed the presence of fibroblast proliferation, collagen formation, angiogenesis and epithelialization.
Results

Table 5.1. Effect of crude methanolic leaf extract on excision wound model

<table>
<thead>
<tr>
<th>Group (n) Treatment</th>
<th>% of closure of excision wound area (original wound area 250 mm²)</th>
<th>Epithelialization in days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4th Day</td>
<td>8th Day</td>
</tr>
<tr>
<td>Control</td>
<td>246.22±0.34**</td>
<td>208.31±0.35**</td>
</tr>
<tr>
<td>Nitrofurazone</td>
<td>244.37±0.31**</td>
<td>187.49±0.59**</td>
</tr>
<tr>
<td>Methanolic leaf extract</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>244.43±0.38**</td>
<td>201.60±0.56**</td>
</tr>
<tr>
<td>200</td>
<td>244.02±0.51**</td>
<td>196.78±0.45**</td>
</tr>
<tr>
<td>300</td>
<td>244.68±0.18**</td>
<td>190.24±0.57**</td>
</tr>
</tbody>
</table>

Value are mean ± SE, n = 6 in each group
** significant at p<0.01
* significant at p<0.05 are compare to control
### Table 5.2. Effect of crude methanolic bark extract on excision wound model

<table>
<thead>
<tr>
<th>Group (n) Treatment</th>
<th>% of closure of excision wound area (original wound area 250 mm²)</th>
<th>Epithelialization in days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4&lt;sup&gt;th&lt;/sup&gt; Day</td>
<td>8&lt;sup&gt;th&lt;/sup&gt; Day</td>
</tr>
<tr>
<td>Control</td>
<td>246.22±0.34**</td>
<td>208.31±0.35**</td>
</tr>
<tr>
<td>Nitrofurazone</td>
<td>244.48±0.26**</td>
<td>187.44±0.38**</td>
</tr>
<tr>
<td>Methanolic bark extract</td>
<td>100</td>
<td>245.01±0.17**</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>243.48±0.35**</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>244.41±0.24**</td>
</tr>
</tbody>
</table>

Value are mean ± SE, n = 6 in each group
** significant at p<0.01
* significant at p<0.05 are compare to control
### Table 5.3. Effect of 13-labdene-2,3,8,15-tetrol on excision wound model

<table>
<thead>
<tr>
<th>Group (n) Treatment</th>
<th>% of closure of excision wound area (original wound area 250 mm²)</th>
<th>Epithelialization in days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4&lt;sup&gt;th&lt;/sup&gt; Day</td>
<td>8&lt;sup&gt;th&lt;/sup&gt; Day</td>
</tr>
<tr>
<td>Control</td>
<td>246.22±0.34**</td>
<td>208.31±0.35**</td>
</tr>
<tr>
<td>Nitrofurazone</td>
<td>244.37±0.31**</td>
<td>187.49±0.59**</td>
</tr>
<tr>
<td>13-labdene-2,3,8,15-tetrol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>244.00±0.38**</td>
<td>210.60±0.66**</td>
</tr>
<tr>
<td>200</td>
<td>244.02±0.51**</td>
<td>200.78±0.35**</td>
</tr>
<tr>
<td>300</td>
<td>244.66±0.18**</td>
<td>196.24±0.27**</td>
</tr>
</tbody>
</table>

Value are mean ± SE, n = 6 in each group  
** significant at p<0.01  
* significant at p<0.05 are compare to control
Table 5.4. Effect of oral administration of crude methanolic leaf extract on resutured incision wound model

<table>
<thead>
<tr>
<th>Group (n) Treatment</th>
<th>Breaking strength (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>361.23±0.35**</td>
</tr>
<tr>
<td>Nitrofurazone</td>
<td>584.99±0.60**</td>
</tr>
<tr>
<td>Methanolic leaf extract</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>463.42±0.44**</td>
</tr>
<tr>
<td>200</td>
<td>492.17±0.50**</td>
</tr>
<tr>
<td>300</td>
<td>530.59±0.36**</td>
</tr>
</tbody>
</table>

Value are mean ± SE, n = 6 in each group
** significant at p<0.01 ; * significant at p<0.05 are compare to control

Table 5.5. Effect of oral administration of crude methanolic bark extract on resutured incision wound model

<table>
<thead>
<tr>
<th>Group (n) Treatment</th>
<th>Breaking strength (g)</th>
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<tr>
<td>Control</td>
<td>365.43±0.46**</td>
</tr>
<tr>
<td>Nitrofurazone</td>
<td>584.95±0.60**</td>
</tr>
<tr>
<td>Methanolic bark extract</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>450.51±0.47**</td>
</tr>
<tr>
<td>200</td>
<td>478.22±0.39**</td>
</tr>
<tr>
<td>300</td>
<td>513.24±0.51**</td>
</tr>
</tbody>
</table>

Value are mean ± SE, n = 6 in each group
** significant at p<0.01 ; * significant at p<0.05 are compare to control

Table 5.6. Effect of oral administration of 13-labdene-2,3,8,15-tetrol, pure compound on resutured incision wound model

<table>
<thead>
<tr>
<th>Group (n) Treatment</th>
<th>Breaking strength (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>361.23±0.35**</td>
</tr>
<tr>
<td>Nitrofurazone</td>
<td>584.95±0.60**</td>
</tr>
<tr>
<td>13-labdene-2,3,8,15-tetrol</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>433.42±0.44**</td>
</tr>
<tr>
<td>200</td>
<td>462.17±0.50**</td>
</tr>
<tr>
<td>300</td>
<td>526.59±0.36**</td>
</tr>
</tbody>
</table>

Value are mean ± SE, n = 6 in each group
** significant at p<0.01 ; * significant at p<0.05 are compare to control
Table 5.7. Effect of oral administration of crude methanolic leaf extract on dead space wound model

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dry weight of granular tissue (mg/100gm)</th>
<th>Tensile breaking strength in gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>64.56± 0.30**</td>
<td>360.90± 0.28**</td>
</tr>
<tr>
<td>Nitrofurazone</td>
<td>81.51± 0.45**</td>
<td>585.52± 0.31**</td>
</tr>
<tr>
<td>Methanolic leaf extract</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>68.45± 0.23**</td>
<td>460.55± 0.47**</td>
</tr>
<tr>
<td>200</td>
<td>72.52± 0.32**</td>
<td>487.40± 0.28**</td>
</tr>
<tr>
<td>300</td>
<td>78.24± 0.46**</td>
<td>521.36± 0.49**</td>
</tr>
</tbody>
</table>

Value are mean ± SE, n = 6 in each group  
** significant at p<0.01 ; * significant at p<0.05 are compare to control

Table 5.8. Effect of oral administration of crude methanolic bark extract on dead space wound model

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dry weight of granular tissue (mg/100gm)</th>
<th>Tensile breaking strength in gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>64.46± 0.42**</td>
<td>360.90± 0.40**</td>
</tr>
<tr>
<td>Nitrofurazone</td>
<td>81.64± 0.38**</td>
<td>585.52± 0.57**</td>
</tr>
<tr>
<td>Methanolic bark extract</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>60.58± 0.47**</td>
<td>454.52± 0.35**</td>
</tr>
<tr>
<td>200</td>
<td>68.62± 0.44**</td>
<td>479.27± 0.52**</td>
</tr>
<tr>
<td>300</td>
<td>71.21± 0.32**</td>
<td>516.30± 0.23**</td>
</tr>
</tbody>
</table>

Value are mean ± SE, n = 6 in each group  
** significant at p<0.01 ; * significant at p<0.05 are compare to control

Table 5.9. Effect of oral administration of 13-labdene-2,3,8,15-tetrol, pure compound on dead space wound model

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dry weight of granular tissue (mg/100gm)</th>
<th>Tensile breaking strength in gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>64.56± 0.30**</td>
<td>360.90± 0.28**</td>
</tr>
<tr>
<td>Nitrofurazone</td>
<td>81.51± 0.45**</td>
<td>585.52± 0.31**</td>
</tr>
<tr>
<td>13-labdene-2,3,8,15-tetrol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>65.35± 0.23**</td>
<td>449.55± 0.47**</td>
</tr>
<tr>
<td>200</td>
<td>69.52± 0.32**</td>
<td>482.50± 0.28**</td>
</tr>
<tr>
<td>300</td>
<td>76.42± 0.46**</td>
<td>518.26± 0.49**</td>
</tr>
</tbody>
</table>

Value are mean ± SE, n = 6 in each group  
** significant at p<0.01 ; * significant at p<0.05 are compare to control
Wound healing activity

Among three animal models viz., excision, incision and dead space wound models, the *E. mallotiformis* methanolic leaf extracts shows significant wound healing activity, when compared to bark and isolated pure compound, 13-labdane-2,3,8,15-tetrol.

The studies on excision wound healing model showed that, there a significant wound healing activity was observed in both groups of animals treated with methanolic leaf extract and its isolated pure compound. The period of epithelialisation and percentage of wound contraction due to the different concentration of methanolic leaf extracts of *E. mallotiformis* are shown in the Table 5.1. Among the three different concentration of methanolic leaf extract, 300 mg/kg concentration shows significant reduction of the wound area in all the assessment days viz., 4th, 8th, 12th, 16th days (244.68±0.18, 190.24±0.57, 102.76±0.44, 50.69±0.49), similar to that of standard drug Nitrofurazone (Table 5.1). But decrease wound healing activity was observed in the control animals for all assessment days viz., 4th, 8th, 12th, 16th days (246.22±0.34, 208.31±0.35, 135.32±0.35, 74.24±0.45) respectively. The time required for complete epithelialization of the excision wound is an important parameter to assess the wound healing activity. It was observed that, mean time taken for complete epithelialization of the excision wound in methanolic leaf extract at the concentration of 300 mg/kg treated group and standard drug treated group was about 18th days, whereas, the mean time taken for complete epithelialization of wound in control animals was about 22nd days (Table 5.1).

Among the three different concentration of methanolic bark extract, 300 mg/kg concentration showed, moderate reduction of the wound area in all the assessment days viz., 4th, 8th, 12th, 16th days (244.41±0.24, 190.46±0.37, 105.43±0.50, 55.72±0.48),
similar to that of standard drug, Nitrofurazone (Table 5.2). But, decrease wound healing activity was obtained in the control animals for all assessment days viz., 4th, 8th, 12th, 16th days (246.22±0.34, 208.31±0.35, 135.32±0.35, 74.24±0.45) respectively. The time required for complete epithelialization of the excision wound is an important parameter to assess the wound healing activity. It was observed that, mean time taken for complete epithelialization of the excision wound in methanolic bark extract, at the concentration of 300mg/kg treated group and standard drug treated group was about 19th days, whereas, the mean time taken for complete epithelialization of wound in control animals was about 22nd days (Table 5.2).

Similarly, in isolated pure compound, 13-labdene-2,3,8,15-tetrol shows significant reduction of the wound area in 300 mg/kg concentration for all the assessment days viz., 4th, 8th, 12th, 16th days (244.66±0.18, 196.24±0.27, 100.76±0.44, 48.69±0.54), similar to that of standard drug, Nitrofurazone (Table 5.3). But, decrease wound healing activity was observed in the control animals for all assessment days viz., 4th, 8th, 12th, 16th days (246.22±0.34, 208.31±0.35, 135.32±0.35, 74.24±0.45) respectively. The time required for complete epithelialization of the excision wound, is an important parameter to assess the wound healing activity. It was observed that, mean time taken for complete epithelialization of the excision wound with 13-labdene-2,3,8,15-tetrol at the concentration of 300 mg/kg treated group and standard drug treated group was about 18th days, whereas, the mean time taken for complete epithelialization of wound in control animals was about 22nd days (Table 5.3).

In the incision model, an incision wound made at paravertebral region of the rats was sutured. Important parameters employed in the assessment of incision wound is of
tensile strength, which can be measured by the skin breaking strength of incised wound, on 10th post wound day using tension meter. The experimental rats with incision wound model, sutured incision wound and desutured incision wound healed animals were subjected to tensile strength evaluation.

Among the crude extracts of leaf and bark and isolated pure compound, methanolic leaf extract exhibit significant activity, compare to methanolic bark and isolated pure compound of E. mallotiformis. The results of methanolic leaf extract, on incision wound healing activity were represented in Table 5.4. Among the three different concentration of methanolic leaf extract, 300 mg/kg concentration showed significant tensile strength value 10th post wound healing day (530.59±0.36 gm), similar to that of standard drug, Nitrofurazone (Table 5.4), but, decrease skin breaking strength was obtained in the control animals on 10th post wound healing day (361.23±0.35 gm). The crude methanolic bark extract showed considerable tensile strength at 300 mg/kg 513.24±0.51 gm (Table 5.5) on 10th post wound healing day. Among the three different concentration of isolated pure compound, 13-labdane-2,3,8,15-tetrol, 300 mg/kg concentration shows significant tensile strength on 10th post wound healing day (526.59±0.36 gm), similar to that of standard drug, nitrofurazone (Table 5.6). But, decrease skin breaking strength was obtained in the control animals, on 10th post wound healing day (361.23±0.35 gm).

The effect of crude extracts and their constituents on dead space wound model was assessed, by the increase in the weight of granulation tissue and increase in the tensile strength. The animals treated with methanolic leaf extract of E. mallotiformis, exhibited high wound healing property, than methanolic bark and isolated pure
compound, 13-labdene-2,3,8,15-tetrol. The results are depicted in the Table 5.7, 5.8 and 5.9.

The animals treated with methanolic leaf extract of *E. mallotiformis* at the concentration 300 mg/kg, exhibits highest wound healing property, as it is revealed by significant increased dry weight of granular tissue (78.24±0.46) and tensile breaking strength (521.36±0.49) (Table 5.7). The Nitrofurazone, showed significant granular tissue dry weight (81.64± 0.38) and tensile breaking strength (585.52±0.57) when compare to all the three concentration of methanolic leaf extract.

Moderate wound healing activity was observed in the animals treated, with methanolic bark extract. The animals received 300mg/kg concentration shows increased dry weight of granular tissue (71.21±0.32) and tensile breaking strength (516.30±0.23), when compare to control group (Table 5.8).

Significant wound healing property was observed in the isolated pure compound, 13-labdene-2,3,8,15-tetrol. The group of animals received, 300 mg/kg concentration showed increased dry weight of granular tissue (76.42±0.46) and tensile breaking strength of (518.26±0.49 gm) (Table 5.9), similar to that of standard drug Nitrofurazone showed dry weight of granular tissue (81.64±0.38) and (585.52± 0.57) tensile breaking strength respectively.

**Histological observation**

The histological studies of the granulation tissues of the control group of animals, showed more number of necrotic cells, with insignificant epithelialization, low rate of collagen formation and increased accumulation of macrophages at the site of injury.
The granulation tissue of 300 mg/kg methanolic extract treated animal group show less number of necrotic cells with significant epithelialisation increased rate of collagen formation, decreased accumulation of macrophages at the site of injury (Plate-5.4B), when compare to the control group. In the standard drug treated group of animals showed less number of necrotic cells with significant epithelialization and increased rate of collagen formation decrease the accumulation of macrophages at the site of injury. Overall study suggest that methanolic leaf extract and isolated pure compound showed significant wound healing activity compared to standard drug (Plate-5.4C).

Discussion

Wound healing activity is a complex and dynamic process of restoring cellular structures and tissue layer in damaged tissue as closely as possible to its normal state. Wound contraction is a process, which occurs throughout the healing process, commencing in the fibroblastic stage whereby, the area of the wound undergoes wound healing shrinkage (Douglas and Alan, 2003).

We have presented a multi scale modeling framework, which allows us to analyze the effect of different factors on wound healing, such as, contraction of cutaneous wound, its tensile strength, collagen alignment, strength of granuloma tissue and scar formation during dermal wound healing. Therefore, in order to examine the above mentioned parameters, three different types of wounds were infected on the experimental rats to assess the healing efficiency of different concentration of methanolic leaf and bark extracts and the isolated constituent, 13-labdane-2,3,8,15-tetrol from methanolic leaf extract of E. mallotiformis. The standard drug Nitrofurazone is used as a standard
reference to assess the healing effects of extracts and the pure compound against the control.

The results of the present study clearly indicated that, the methanolic leaf extract and its constituent, enhanced healing of all the three types of cutaneous wounds. Application of ointments base prepared from methanolic leaf extract, displayed significant wound healing activity in excision wound (50.69±0.49). The healing time required for complete epithelialization of the excision wound was found to be much earlier (18\textsuperscript{th} post wound day) and it was on par with that of the standard reference drug, Nitrofurazone. While, the methanolic bark extract treated group of animals also exhibited moderate wound healing activity (55.72±0.48), when compared to the control group of animals (74.24±0.45), in this, the duration of epithelialization was delayed by 1 day. The complete epithelialization in control group occurred on 22\textsuperscript{nd} post wounding day. The isolated pure compound, 13-labdene-2,3,8,15-tetrol exhibited significant wound healing activity (48.69±0.54), which is similar to methanolic leaf extract and standard drug. As the rate of wound contraction was faster in standard drug (43.81±0.47) treated group of animals and the complete epithelialization of the excision wound was observed on 18\textsuperscript{th} post wound day. The above results are comparable with the findings of Singh \textit{et al.} (2005), Manjunatha \textit{et al.} (2005) and Kumaraswamy \textit{et al.} (2007).

In incision wound model, the breaking strength of the granulation tissue increases proportionately, with the collagen deposition. The tensile strength of the wound is determined by the rate of collagen synthesis and maturation process involving inter and intra molecular cross linking of collagen fibrils. The breaking strength of a healing wound can be measured practically, by the minimum amount of force required to
disarticulate it. In the beginning, a wound will be having little breaking strength, that increases proportionately as collagen deposition increases and cross linking are formed between collagen fibers.

In the present study, using a linear resutured incision model, the wound breaking strength was determined on 10th post wounding day. By treating the wounded animals, with methanolic leaf extract, it was observed that, tensile breaking strength of tissue treated with methanolic leaf, at 300 mg/kg showed, significant result (530.59±0.36), when compared with a standard drug (584.99±0.60). The isolated pure compound, 13-labdene-2,3,8,15-tetrol, from methanolic leaf extract also exhibited significant tensile strength (526.59±0.36), when compare with control and standard group of animals.

Dead space wound model provides an opportunity to study the effect on the granulation and collageneration of the healing process. Such wound models has been employed for the quantitative study on wound healing, such as, granuloma breaking strength and hydroxylprolin content (Patil and Kulakarni, 1984).

Granulation tissue formed in the final part of the proliferation phase is primary composed of fibroblasts, collagen, edema and new small blood vessels, collagen is the major component, which strengthens and supports extra cellular tissue. The increase in the granulation tissue weight, suggested higher protein content and increase in the hydroxyproline content of the granulation tissue, indicating increased collagen turnover (Gupta and Gupta, 1985). Its measurement could be used as an index for collagen turnover (Madhura and Shushma, 2003). Gain in granuloma breaking strength, indicates increased collagen maturation by increased cross-linking. Collagen mainly composes of
the amino acid, hydroxyproline. So, the estimation of hydroxyproline content gives the net rate of synthesis and deposition of collagen in healing wound (Kumar et al., 2006).

In the present study, healing efficiency of methanolic leaf extract and isolated constituents on the dead space wound models were evaluated by assessing the weight of granuloma tissue, by estimating it's breaking strength. Among the treated animals, the response was showed to be best in methanolic leaf extract treated animals, in this case, dry weight of granular tissue was 78.24±0.46 and tensile breaking strength 521.36±0.49. The isolated pure compound, 13-labdone-2,3,8,15-tetrol showed dry weight of granular tissue of 76.42±0.46 and tensile breaking strength of 518.26±0.49 gm. Both the methanolic leaf extract and its constituents, significantly augmented the breaking strength of granuloma tissue harvested on 10\(^{th}\) day. This may be due to the enhanced collagen maturation, by increased cross-linking of collagen fibers. But, the methanolic bark extract treated animals, showed insignificant value in the formation of granular tissue and tensile strength (71.21±0.32 and 516.30±0.23). The above results are comparable with the findings of Singh et al. (2005), Manjunatha et al. (2005) and Kumaraswamy et al. (2007).

In histological inspection of the granuloma tissues, showed the infiltration of fibroblast and macrophages in the subcutis it was significantly greater in the untreated animals. Furthermore, there was significantly decreased epithelialization and lesser collagen regeneration, indicates the incomplete wound healing. The section of the granuloma tissue harvested from the animals, supplemented with methanolic leaf extract of \textit{E. mallotiformis} and isolated pure compound, 13-labdone-2,3,8,15-tetrol, provides, further evidences on their wound healing efficiency. The section of granuloma tissue obtained from methanolic leaf extract treated animals showed complete epithelialization,
complete fibrosis and appearance of few macrophages and significantly increased collagen formation. In case of animals treated with methanolic bark extract of *E. mallotiformis*, moderate deposition of collagen was observed as compared to the control.

There are reports that, the plants having antioxidant property, would also enhance wound healing activity (Shirwaikar et al., 2003). Hence, in our study, better wound healing activity was observed in methanolic extract of *E. mallotiformis*. The methanolic extract contains flavanoids, which are strong antioxidants. This fact may convince the better wound healing potency of the methanolic extract.
Plate -5.1

Photographs showing different stages of wound healing of treated animals in excision wound model

A - Excision wound of methanolic leaf extract treated animals on Day 1\textsuperscript{st}
B - Excision wound of methanolic leaf extract treated animals on Day 4\textsuperscript{th}
C - Excision wound of methanolic leaf extract treated animals on Day 8\textsuperscript{th}
D - Excision wound of methanolic leaf extract treated animals on Day 12\textsuperscript{th}
E - Excision wound of methanolic leaf extract treated animals on Day 16\textsuperscript{th}
F - Complete epithelialization on 18\textsuperscript{th} post wound day with methanolic leaf extract treated animal
Plate - 5.2

Photographs showing method of performing incision wound on test animals

A - Making incision wound on paravertebral region
B - Suturing incision wound on paravertebral region
C - Assessing tensile strength using tensiometer.
Plate - 5.3

Photographs showing method of performing dead space wound on test animals

A - Dead space wounds created in subcutaneous and implantation of sterilized cylindrical grass pith.

B - Suturing after implantation of sterilized cylindrical grass pith.

C - Removing grass pith after formation of granulation tissue on 10th post wounding day.
Plate - 5.4

Photographs showing microscopic views of granular tissue of experimental animals

A - Section of the granulation tissue of the control animal showing more macrophages (Blue arrow) and less collagen formation (White arrow) (40X)

B - Section of the granulation tissue of methanolic leaf extract treated animal showing collagen fibers (White arrow) and more macrophages (Blue arrow) (40X)

C - Section of the granulation tissue of the isolated pure compound 13-labdane-2,3,8,15-tetrol treated animal showing collagen fibers (White arrow) and more macrophages (Blue arrow) (40X)
References


