Evaluation of wound healing and antimicrobial activity of *Lannea coromandelica* (Hoult) Merr

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ABSTRACT

Ethanol and acetone extracts of *Lannea coromandelica* (Hoult) merr (Anacardiaceae) barks in the form of simple ointments screened for wound-healing activity by incision and excision wound on male Wister rats. In excision wound model, more than 95% wound healing was recorded in treated groups by 16 days of post surgery, whereas only 85.40% was observed in control group. Significant (P<0.01) epithelialization was observed in the groups treated with both ethanol and acetone extract ointments. In incision wound model higher breaking strength were observed 82.43%, 63.77% in ethanol and acetone extracts treated groups respectively suggested higher collagen re-deposition than the control group. Framycetin sulphate (1%) was used as standard in both incision and excision wound models. Finally, histopathological studies confirmed the wound healing properties. The results shown that *Lannea coromandelica* extract has potent wound healing activity by in terms of significant wound contraction and increased tensile strength. Since wound healing is severely influenced by microbial infection, this study was extended to evaluate antimicrobial activity apart from wound healing activity. The antimicrobial activity was determined by disc diffusion method.

Keywords: Wound healing; *Lannea coromandelica*; Tensile strength; Antimicrobial activity.

INTRODUCTION

Wound healing is a dynamic process involving biochemical and physiological phenomena that behave in a harmonious way in order to guarantee tissue restoration. [1] *Lannea coromandelica* belongs to family Anacardiaceae is a deciduous tropical tree distributed throughout India, Bangladesh and some other tropical countries. Five dihydroflavonols were isolated and identified from the stem barks of *Lannea coromandelica*. [2] The bark is useful in cuts, wounds, bruises, ulcers, ophthalmia, gout, ulcerative stomatitis, odontalgia, sprains, diarrhea and dysentery, and leaves in elephantiasis, inflammation, neuralgia, sprains and bruises. The fruits paste of *Lannea coromandelica* therapeutically used for bone fractures by tribes in eastern Ghat of Andhra Pradesh. [3] The pharmacological properties of extract of *Lannea coromandelica* stem barks were screened for anti-inflammatory [4], hypotensive [3], and cytotoxicity effects. [5] The wound healing activities of plants have since been explored in folklore. The significant successes recorded have led to investigation into medicinal plants with a view to confirming those acclaimed properties. In the present study *Lannea coromandelica* was found to be effective in healing of wounds. The extracts ointment were prepared and studied for its wound healing property on different models. The wound healing process may be hampered by microbial infection. Records have it that different parts of plants used for wound healing contain some active principles or components that possess antimicrobial function. [6] Hence this study under taken to evaluate the antimicrobial activity of *Lannea coromandelica* apart from wound healing activity.

MATERIALS AND METHODS

Plant material

Barks of *Lannea coromandelica* (Hoult) merr were collected from C.C.D.-Gram Mooligai Institute, Thottiankulam, Madurai with the help of Dr.P.Saravanan (Program coordinator). The sample specimens of *Lannea coromandelica* was identified and authenticated by Dr. D. Stephan, Taxonomist, American college, Madurai. The barks were shade dried under room temperature for a period of one month, and coarsely powdered by using mixer grinder. The powder was subjected to Continuous extraction in a soxhlet apparatus using ethanol and acetone for 72 hours. The extracts were concentrated to a dry mass by concentrating on rotary evaporator, and keeping it in decicator. The percentage yield of ethanol and acetone extracts were found to be 11.06% and 9.22% respectively.

Drug formulation

The powdered residues of ethanolic and acetone extracts of *Lannea coromandelica* were mixed with simple ointment base to make 10% w/w ointment. The fusion method was employed in the preparation of the medicated Ointments. The required quantity of the ointment base was weighed and melted at a temperature of about 70°C in a hot water bath. The designated quantities of the extract(s) were respectively added to the melted base at 40°C and the mix, stirred gently and continuously until a homogenous dispersion is obtained. The Ointments were packed in sterile labeled jar and stored in a cool place.

Animals and Microbial strains

Male wister rats with no prior drug treatment weighing 150-200 gm were used for the study. Animals were housed under...
standard laboratory conditions at temperature (22±3°C) and relative humidity (45-55%) with 12:12 light and dark cycle. The animals were fed with standard pellet diet (Amruth animal feed, Bangalore) and water *ad libitum*. The animals were acclimatized to laboratory hygienic conditions for 10 days before starting the experiment. The animal studies were approved by Institutional Animal Ethical Committee (Approval no. 890/ac/05/CPCSEA) of the Ultra College of Pharmacy Madurai. The test micro organisms used for the antimicrobial screening were 4 bacteria (2 G<sup>+</sup> and 2 G<sup>-</sup>) *Bacillus cereus* MTCC 430, *Staphylococcus aureus* MTCC 96, *Escherichia coli* MTCC 443, *Proteus vulgaris* MTCC 1771, and a systemic fungi *Aspergillus niger* MTCC 872. These organisms were procured from Institute of Microbial Technology (IMTEC-CSIR), Chandigarh, India.

### WOUND HEALING ACTIVITY

#### Grouping of animals

For incision and excision wound model the animals weighed between 150-200 gm were divided into four groups in each model consisting of six animals as follows: Group I - simple ointment base BP; Group II - 1% Framycetin sulphate cream (Aventis); Group III - 10% w/w ethanolic extract ointment of *Lannea coromandelica* (EOLC) and Group IV - 10% w/w acetone extract ointment of *Lannea coromandelica* (AOLC).

#### Excision wound model

An incision was made on the dorsal thoracic region 1cm away from vertebral column and 5cm away from the area using a round seal of 2.5cm diameter on the anaesthetized rats. The skin of impressed area was excised to full thickness to obtain a wound area of about 500mm<sup>2</sup> diameters. The full thickness excised wound was created by using toothed forceps, surgical blade and pointed scissors. Homeostasis was achieved by blunting the wound with cotton swab soaked in normal saline. The entire wound was kept undressed. The simple ointment base, standard drug 1% Framycetin sulphate cream, EOLC and AOLC were applied topically to the control group, standard group and treatment groups respectively till the wound was completely healed.[9]

#### Incision wound model

All the animals were anaesthetized and the dorsal furs of the animals were shaved before one day of experiment. In this model, 1.5 cm from midline, 6cm long Para vertebral incision was made through full thickness of skin of either side of the vertebral column of the rat. All the groups were treated in same manner as mentioned in case of excision wound model, the both edges kept together and stitched with black silk surgical thread (no.000) and a curved needle (no.11). Stitching made at 0.5cm apart. The entire wound was kept undressed. The 1gm of respective ointments was topically applied daily for 9days. On 9<sup>th</sup> day the sutures were removed and the skin breaking strength of the wound was measured on 10<sup>th</sup> day by using tensiometer.[9,10]

#### Wound healing evaluation parameters

##### Wound contraction and epithelialization time

In excision wound model the wound healing property was evaluated by wound contraction percentage and wound closure time. Contractions, which contribute for wound closure in the first 2 weeks, were studied. The area of wound was created on transparent paper and calculated using graph sheet from the healed wound, a specimen sample tissue was isolated from each rat for histopathological examination. The epithelialization time was measured from initial day.[8-10]

#### Measurement of tensile strength

Tensile strength is a 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. Usually wound healing agents promote a gain in tensile strength. In incision wound model the Sutures were removed on 9<sup>th</sup> day after wound creation and the tensile strength was measured on the 10<sup>th</sup> day with the help of tensiometer, which is based on method of Kuwano.[7] In this method wound-breaking strength was measured as the weight of water at the time of wound breaking per area of the specimen. The tensile strength was calculated by following formula:

\[
\text{Tensile Strength} = \frac{\text{Breaking Strength (g)}}{\text{Cross section Area of skin (mm}^2\text{)}}
\]

#### Histopathological Studies

Wound skin tissue specimen from treated and untreated rats were collected after complete healing of the incision and excision wound model in 10% buffered formalin and after usual processing, 5µm thick section were cut and stained with haematoxylin and eosin. Sections were qualitatively assessed under light microscope and observed in respect of fibroblast proliferation, collagen formation, angiogenesis, epithelialization, neovascularisation and macrophages.[9,10]

#### Statistical analysis

All treated groups were compared with the control group and results were expressed as a mean ±SD of 6 animals in each group. The results were analyzed statistically using one-way ANOVA followed by Dennett test, P<0.05 were considered as significant

#### Antimicrobial Activity

The disc diffusion method was used to evaluate the antimicrobial activity.[11] The Molten Muller Hinton Agar (MHA) for bacteria incubated over night at 37°C and Potato Dextrose Agar (PDA) for fungus incubated 28°C for 72 hrs were used as inoculum’s. Standard antibiotics disc (6mm in diameter) Kanamycin 30µg/ml and Clotrimazole 10µg/ml were used as positive control for bacteria and fungus respectively, plant extract discs (6mm in diameter) were impregnated with 10µl of 100mg/ml of respective ethanol extract (EELC) and acetone extract (AELC) dissolved in dimethyl sulphoxide. All discs were placed on seeded agar.[8]

#### RESULTS

Significant wound healing activity was observed in both the EOLC and AOLC treated groups. The significant percentage of wound concentrations 99.32%, 97.11% and 95.95% were found for standard, EOLC and AOLC treated groups respectively in excision wound model. While in control animals it was only 85.40% on day 16. The time required for complete epithelialization was found 20.33±3.01 days for control animals, in standard treated animals it was 15.5±1.05days. In EOLC and AOLC treated groups the epithelializations were found on 16.17±2.14 days respectively in excision wound model.

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*R.Sathish et al. / Journal of Pharmacy Research 2010, 3(6),1225-1228*

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Table 1: Effect of *Lannea coromandelica* barks on Wound Area of Excision wound Model

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Wound Area (mm²) Day – 1</th>
<th>Wound Area (mm²) Day – 4</th>
<th>Wound Area (mm²) Day – 8</th>
<th>Wound Area (mm²) Day – 12</th>
<th>Wound Area (mm²) Day – 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>555.66±4.4</td>
<td>413.83±21.91</td>
<td>301.5±15.19</td>
<td>156.5±12.53</td>
<td>81.17±4.06</td>
</tr>
<tr>
<td>II</td>
<td>512±2.7</td>
<td>336.6±3.28</td>
<td>176.8±7.83</td>
<td>40.83±0.79</td>
<td>3.5±8.57</td>
</tr>
<tr>
<td>III</td>
<td>542±1.98</td>
<td>378.3±16.38</td>
<td>217.67±24.05</td>
<td>59.33±8.55</td>
<td>15.67±18.09</td>
</tr>
<tr>
<td>IV</td>
<td>547±2.39</td>
<td>343.3±13.71</td>
<td>206.33±14.92</td>
<td>66.5±10.31</td>
<td>22.17±24.58</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD (n = 6)

**=P<0.001, *=P<0.01, compared with control group

Table 2: Effect of *Lannea coromandelica* barks on Epithelialization Period and Percentage Closer of Excision Wounds

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Percentage Closer of Excision Wound Area Day – 4</th>
<th>Day – 8</th>
<th>Day – 12</th>
<th>Day – 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>25.67</td>
<td>45.74</td>
<td>71.84</td>
<td>85.40</td>
</tr>
<tr>
<td>II</td>
<td>34.44</td>
<td>65.46</td>
<td>92.03</td>
<td>99.32</td>
</tr>
<tr>
<td>III</td>
<td>30.20</td>
<td>59.93</td>
<td>89.05</td>
<td>97.11</td>
</tr>
<tr>
<td>IV</td>
<td>37.23</td>
<td>61.28</td>
<td>87.84</td>
<td>95.95</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD (n = 6)

*=P<0.01, compared with control group

Table 3: Effect of *Lannea coromandelica* Barks on Breaking Strength and Tensile Strength Incision Wound Model

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Breaking Strength (g) Day – 1</th>
<th>Breaking Strength (g) Day – 4</th>
<th>Breaking Strength (g) Day – 8</th>
<th>Breaking Strength (g) Day – 12</th>
<th>Breaking Strength (g) Day – 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>240.17±8.08</td>
<td>-</td>
<td>120±0.01</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>459.67±5.79*</td>
<td>91.40</td>
<td>230±0.32*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>III</td>
<td>438±15.94*</td>
<td>82.43</td>
<td>219±0.08*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IV</td>
<td>393.3±20.41*</td>
<td>63.77</td>
<td>197±0.41*</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD; n = 6.

*=P<0.001, compared with control group

Table 4: Antimicrobial activity of *Lannea coromandelica* Barks on different microbes

<table>
<thead>
<tr>
<th>S.no</th>
<th>Microorganisms</th>
<th>Inhibition zone in diameter (mm)</th>
<th>Standards Kanamycin 30µg/disc</th>
<th>Standards Clotrimazole 10µg/disc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>EELC</td>
<td>AELC</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td><em>Bacillus cereus</em> MTCC 430</td>
<td>20</td>
<td>19</td>
<td>34</td>
</tr>
<tr>
<td>2</td>
<td><em>Staphylococcus aureus</em> MTCC 96</td>
<td>26</td>
<td>22</td>
<td>32</td>
</tr>
<tr>
<td>3</td>
<td><em>Escherichia coli</em> MTCC 441</td>
<td>13</td>
<td>14</td>
<td>26</td>
</tr>
<tr>
<td>4</td>
<td><em>Proteus vulgaris</em> MTCC 1771</td>
<td>12</td>
<td>11</td>
<td>27</td>
</tr>
<tr>
<td>5</td>
<td><em>Aspergillus niger</em> MTCC 872</td>
<td>21</td>
<td>22</td>
<td>26</td>
</tr>
</tbody>
</table>

Fig1: Histopathology of skin for Incision wound models

Fig2: Histopathology of skin for Excision wound models

and 459.67±5.79gm respectively as compared to control group, breaking strength of AOLC treated animals was 393.33 ± 20.41gm. The breaking strength of treated groups on day 10 was found to be significant P<0.001 when compared with control group (Table3). Histology of wound tissue of control animals showed the presence of acute inflammatory cells, fibroblastic connective tissue and very little number of blood vessels. The lesser epithelialization and lesser collagen formation indicated incomplete healing of wound. Where as, in the sections of Framycetin treated animals increased collagen deposition was observed. The sections of granuloma tissues of EOLC and AOLC treated animals showed moderate epithelialization, fibrosis, collagen formation and increased number of blood vessels (Fig 1,2). Ethanol and acetate extracts of *Lannea coromandelica* bark had shown inhibition effect on the growth of all the organisms tested. Zone of inhibition for ethanol extract ranges between 12 to 26mm and for acetone extract was from 11 to 22mm (Table 4).

**DISCUSSION**

Wound healing process consists of different phases such as granulation, collagenation, collagen maturation and scar maturation which are concurrent but independent to each other. Wound healing is a complex cellular event by which a damaged tissue restored as closely as possible to its normal stage. The healing process depends upon the reparative abilities of the tissue, the type and extent of damage and general state of health of the tissue.[12] The inflammation stage begins immediately after injury, first with vasoconstriction that favors homeostasis and releases inflammation mediators. The proliferative phase is characterized by granulation tissue proliferation formed mainly by fibroblast and the angiogenesis process. The remodeling stage is characterized by reformation and improvement in the components of the collagen fiber that increases the tensile strength.[13] The paste of *Lannea coromandelica* fruits are applied externally for bone fracture as a folk medicine by the adivasis of Eastern Ghats, Andhra Pradesh, India.[3] The plant also used in traditional medicine for leprous and obstinate ulcers, toothache, mouth sores and impotency.[14] Hence in this study two different models were used to assess the effect of herbal ointments on various phases. The results showed that ethanol and acetone extract ointments possesses a definite pro healing action. This was demonstrated by a significant increase in the rate of wound contraction, tensile strength and enhanced epithelialization of treated groups. When a wound occurs and in exposed to external environment, it is more prone to attack by microbes, which invade through the skin and delay the natural wound healing process. Phytochemical work concluded that both acetone and ethanol extracts of *Lannea coromandelica* contains flavonoids. The dihydroflavonols were isolated from the stem bark of *Lannea coromandelica*. According to previous studies plants *Tephrosia purpurea* and *Lantana camara* contains flavonoids, which have been documented to have antimicrobial and wound healing activity.[10,11] In conclusion, this study confirms the promising wound healing activity of *Lannea coromandelica* stem bark. The external application of both extracts on wound prevents the microbes to invade through the wound.
resulting protection of wound against the infections of various microorganism. This synergistic effect of antimicrobial activity accelerated the wound healing activity. These finding could justify the inclusion of this plant in the management of wound healing.

REFERENCES

2. Tofizzal islam MD and Satoshi Tahara, Dihydroflavonols from Lannea coromandelica, Phytochemistry 54, 2000, 901-907.

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