Wound healing potential of Leathery Murdah, *Terminalia coriacea* (Roxb.) Wight & Arn.

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Abstract

Leathery Murdah (*Terminalia coriacea* {Roxb.} Wight & Arn.) belonging to family Combretaceae is found in dry and warmer parts of Andhra Pradesh and Tamil Nadu, states of India. Traditionally the stem bark of this plant is used to treat callous ulcers. Hence, the present study was undertaken to perform preliminary phytochemical screening and investigate the wound healing potential of *Terminalia coriacea* Stem Bark Methanolic and Aqueous Extracts (TCSBME/TCSBAE) 5% w/w ointments by excision wound model in albino wistar rats using Povidone Iodine 5% w/w ointment as standard. Both the extracts TCSBME & TCSBAE produced significant wound healing effect with p < 0.01 & p < 0.05 respectively. The epithelialization was observed on 17th and 19th day of post-wounding comparable to standard. This provides preliminary evidence of wound healing activity of *T. coriacea*. Further, early fall of scar & recovery of wound with premature development of hair in TCSBME treated group, indicated hair-growth promoting property. Additional studies are required to identify responsible phytochemicals and to ascertain the mechanism of action.

Keywords *Terminalia coriacea*; Leathery Murdah; wound healing.
Introduction

The function of skin is to serve as a protective barrier against the environment. Wounds are physical injuries that result in an opening or breaking of skin. Healing is a complex intricate process initiated in response to an injury that restores the function and integrity of damaged tissues (Agarwal et al., 2009). Healing process can be broadly categorized into three stages, inflammatory phase (consisting the establishment of homeostasis and inflammation), proliferative phase (consisting of granulation, contraction and epithelialization) and finally the remodeling phase which determines the strength and appearance of the healed tissue (Evans, 1980). Wounds are inescapable events in life. Wounds may arise due to physical, chemical or microbial agents in life (Harshmohan, 2005). The prevalence of chronic wounds in the community was reported as 4.5 per 1000 population whereas that of acute wounds is nearly double at 10.5 per 1000 population. Healing of a chronic wound requires care that is patient centered, holistic, interdisciplinary and should be cost effective and evidence based (Gupta et al., 2004).

Majority of world population relies on traditional medicine for their health care needs (Zhang, 1996). Several natural products, plant products which are composed of active principles, like triterpenes, alkaloids, flavonoids and biomolecules have been reported to promote the process of wound healing (Suguna et al., 1999; Sharma et al., 1990; Chitra et al., 1995). Using certain herbs which possess antiseptic, astringent, anti-inflammatory, antimicrobial, antioxidant and bio-stimulatory properties can enhance the rate of wound healing (Somashekar et al., 2006; Sunil et al., 2008). These herbs increase the rate of tissue healing by providing different essential substances, required at various steps of regeneration. These herbs being cheaper and safer than allopathic drugs may be useful in veterinary practice, especially in India where these are found in plenty (Wallis, 2002). Moreover the current methods used to treat chronic wounds include debridement, irrigation, antibiotics, tissue grafts and proteolytic enzymes, which possess major drawbacks and unwanted side effects (Nayak et al., 2009).

Many herbs are used in traditional medicine to cure wounds; Leathery Murdah (*Terminalia coriacea* {Roxb.} Wight & Arn.) is one such plant, which is widely distributed in drier and warmer parts of Andhra Pradesh and Tamil Naidu upto 1350m and in central India. It belongs to family Combretaceae and is called as Tani in Telugu (the regional language). The bark of the plant is traditionally used as cardiac stimulant, in treatment of atonic diarrhoea and callous ulcer (Chetty et al., 2008; Khare, 2007). Literature survey reveals this specie is rich in tannins and phenolic content and exhibits high anti-oxidant activity (Mety and Mathad, 2011). This can be the basis for the medicinal properties of the plant. Considering this observation, we conducted preliminary pharmacological investigations.

In our previous study we performed preliminary phytochemical screening, acute toxicity test and conducted anti-nociceptive studies of *T. coriacea* leaf methanolic extract in mice at 125, 250 & 500 mg/kg, b.w, p.o. The investigation revealed the presence of alkaloids, amino acids, carbohydrates, flavonoids, glycosides, resins, saponins, sterols, tannins, triterpenoids and phenolic compounds in TCLME. Acute toxicity testing of TCLME indicated that the doses up to 2000 mg/kg were safe as there was no mortality and signs of toxicity. Three test doses (125, 250 and 500 mg/kg, p.o) in the range of 1/16th – 1/4th of observed maximum safe dose were subjected to the evaluation of anti-nociceptive property. TCLME at 250 &
500 mg/kg doses demonstrated significant (p< 0.01) analgesic effect against thermal stimulus-induced pain and acetic acid induced writhing in mice (Khan et al., 2011).

In another study we performed preliminary phytochemical screening, acute toxicity and anti-pyretic activity of *T. coriacea* stem bark aqueous extract (TCSBAE) against yeast induced hyperpyrexia model in rats at 125 & 250 mg/kg orally. Acute toxic dose was found to be above 1000 mg/kg. Test extract at 250 mg/kg dose decreased elevated body temperature significantly (p< 0.01) similar to that of standard, Paracetamol 200 mg/kg, p.o, whereas TCSBAE 125 mg/kg showed mild anti-pyretic activity (p<0.05) (Bhatt and Khan, 2010). The present investigation was undertaken to screen wound healing potential of *T. coriacea* stem bark extracts in albino wistar rats.

**Materials and methods**

**Plant Material**

The stem bark of *Terminalia coriacea* (Roxb.) Wight & Arn. belonging to Combretaceae were collected from Tirumala Hills, Chittoor district (A.P). The plant material was identified and authenticated by Dr. K. Madhava Chetty (Assistant Professor, S.V. University, Tirupati, India) & a voucher number 985 was assigned to the reference sample.

**Processing and Storage of Extract**

The stem bark of *Terminalia coriacea* (Roxb.) Wight & Arn. was washed to render it free from dust. The material was then dried under shade for about 5-7 days and weighed. About 1 kg of the dried bark was grinded into a coarse powder using a mechanical grinder and passed through sieve no.40 to get the powder of desired coarseness. The powdered material was then preserved in an air tight container for future use.

**Preparation of Extracts**

(i) **Preparation of Terminalia coriacea (Roxb.) Wight & Arn. Stem Bark Methanolic Extract (TCSBME) by Soxhlet Extraction**

150 gm of the powdered stem bark of *T. coriacea* was extracted in Soxhlet apparatus using methanol (600 ml), as a solvent in 1:4 ratio. The process was continued until the extraction was complete (indicated by fade coloured menstrum). The solvent was distilled off and the extract was concentrated on a water bath. The concentrated extract was then weighed and the percentage yield was calculated. TCSBME was then subjected to preliminary phytochemical screening (Khandelwal, 2004; Kokate et al., 2007; Harborne, 1978).

(ii) **Preparation of Terminalia coriacea (Roxb.) Wight & Arn. Stem Bark Aqueous Extract (TCSBAE) by Decoction**

About 200 gm of the powdered stem bark of *Terminalia coriacea* (Roxb.) Wight & Arn. was moistened by soaking it in 500 ml of distilled water (1: 2.5) and allowed to stand overnight. The material was later boiled for 5 hours with constant stirring at an interval of 1 hour. The material was filtered to obtain a decoction which was later concentrated to a syrupy liquid on a water bath. The concentrated TCSBAE was then weighed and the percentage yield
was recorded. TCSBAE was also subjected to preliminary phytochemical screening (Khandelwal, 2004; Kokate et al., 2007; Harborne, 1978).

**Drug Formulation**

A water soluble ointment base was prepared by mixing 40% of PEG 4000 and 60% of PEG 400 and was employed as control drug. The test drug formulations of TCSBME and TCSBAE were prepared separately by incorporating 5 g of the test extracts in 100 g of water soluble ointment base. Povidone iodine (Betadine ointment 5% w/w) was used as a standard drug for comparing the wound healing potential of TCSBME and TCSBAE.

**Experimental Animals**

Adult male wistar rats weighing 150-200 gm were used to evaluate wound healing activity by excision wound model. The animals were maintained under standard laboratory conditions in polypropylene cages under 12 hr light/dark cycle, controlled temperature (24 ± 2°C), fed with commercial pellet diet and water *ad-libitum* in an animal house approved by Committee for the Purpose and Supervision on Experiments on Animals (Reg no: 1534/PO/a/11/CPCSEA). All the animals were acclimatized to the laboratory environment for 10 days before commencement of the experiments. The experiments were carried out in accordance with the instructions of Institutional Animal Ethical Committee, Anwarul Uloom College of Pharmacy, New Mallepally, Hyderabad 500001, Andhra Pradesh, India.

**Draize Test**

Male albino wistar rats weighing (150-200 gm) with n=6 per group were used in skin irritation test. Two patches each of two square inch area were prepared by shaving the dorsal surface of one rat. Patch made from two layers of light gauze was dipped in solutions containing different concentrations -0% (Control), 2.5%, 5% and 10% of test extracts (TCSBME & TCSBAE) prepared in PG:EtOH (7:3). The animals were immobilized in the special holder during the 24 hrs patch exposure. Upon removal of the patches the animals were observed for any sign of erythema or oedema for a period of 72 hrs. The observations were repeated after 72 hrs (Kaushal et al., 2011).

**Evaluation of Wound Healing Activity**

**Excision Wound Model**

Animals were anesthetized prior to and during creation of the wounds, with intravenous ketamine hydrochloride (120 mg Kg⁻¹ body weight). The rats were inflicted with excision wounds as described by Morton and Malone (1972). The dorsal fur of the animals was shaven with an electric clipper and the anticipated area of the wound to be created were outlined on the back of the animals with methylene blue using a circular stainless steel stencil. A full thickness of the excision wound of circular area of 300 mm² wide and 2 mm deep were created along the markings using toothed forceps, scalpel and pointed scissors. The animals were divided randomly in four different groups (Table 1) as mentioned in the treatm-
Table 1. Experimental Design & Treatment Schedule

<table>
<thead>
<tr>
<th>S. No</th>
<th>Groups</th>
<th>Topical Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I [ - ve Control]</td>
<td>Water soluble ointment base (40% PEG 4000 &amp; 60% PEG 400)</td>
</tr>
<tr>
<td>2</td>
<td>II [Standard]</td>
<td>Povidone iodine (5% w/w) ointment</td>
</tr>
<tr>
<td>3</td>
<td>III [Test - I]</td>
<td>TCSBME ointment (5% w/w)</td>
</tr>
<tr>
<td>4</td>
<td>IV [Test - II]</td>
<td>TCSBAE extract ointment (5% w/w)</td>
</tr>
</tbody>
</table>

ent schedule. The wound closure rate was assessed post-wounding by tracing the wounds on days 0, 2, 4, 8, 12 & 16 using transparent paper and a permanent marker. Change in wound area was calculated, giving an indication of the rate of wound contraction. The wound areas were measured and recorded using graph paper. The day of scar falling without any residual raw wound were considered as period of epithelialization (Nayak et al., 2009).

**Statistical Analysis**

The values are expressed as Mean ± SEM. P < 0.05 was considered significant, denoted by symbol (*). The data was analyzed by One-way Analysis of Variance followed by Dunnett’s multiple comparison post-hoc test using GraphPad Instat version 3.10 for Windows, GraphPad Software, San Diego California USA.

**Results and Discussion**

**Extraction and Preliminary Phytochemical Screening**

The weight of final concentrated semi-solid mass obtained after extraction was found to be 32.3 g and 22 g for TCSBME and TCSBAE respectively corresponding to 23.4% and 11% yield. The former was dark reddish brown while later was light reddish brown in colour. Qualitative phytochemical investigation reveals the presence of phytoconstituents like alkaloids, anthraquinone glycosides, carbohydrates, flavonoids, saponins and tannins. The results of phytochemical screening are summarized in Table 2.

**Draize Test**

No signs of allergy (allergic spots or redness of skin) were observed on rat's skin during the skin irritancy test. There were no cases of wound infection in all the treated groups.

**Excision Wound Model**

Topical application of both the test extracts (TCSBME and TCSBAE) in the form of (5 % w/w) ointments at wound site produced significant wound healing effect. Treated excision wounds showed an increase in rate of wound contraction, leading to faster healing as confirmed by the increased healed area when compared to the control group. The results of present study are shown in Table 3. It indicates that both the test extracts possess wound healing potential and the effects are comparable to that of standard. Among the test extracts, TCSBME (5% w/w) ointment was found to be more effective than TCSBAE (5% w/w) ointment.
Table 2. Results of preliminary phytochemical screening.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Tests</th>
<th>TCSBME</th>
<th>TCSBAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Mayer’s test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>b.</td>
<td>Hager’s test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>c.</td>
<td>Wagner’s test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>d.</td>
<td>Test for tannic acid</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2 (I)</td>
<td>Tannins:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>5% FeCl₃</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>b.</td>
<td>Lead acetate</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>c.</td>
<td>Gelatin solution</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>d.</td>
<td>Bromine water</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>e.</td>
<td>Acetic acid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>f.</td>
<td>Potassium dichromate</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>g.</td>
<td>Dilute iodine solution</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>h.</td>
<td>Dilute HNO₃</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>i.</td>
<td>Dilute NH₄OH &amp; Potassium ferricyanide</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>j.</td>
<td>NH₄OH &amp; AgNO₃ Solution</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>k.</td>
<td>Dilute KMnO₄</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2 (II)</td>
<td>Condensed tannins:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Vanillin HCl Test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>b.</td>
<td>Match stick</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Steroids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Salkowski reaction</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Cardiac glycosides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Keller Killiani test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>Anthraquinone Glycosides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Borntrager’s test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Saponins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Borntrager’s test</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: TCSBME and TCSBAE refer to T. coriacea Stem Bark Methanolic extract and T. coriacea stem bark aqueous extract respectively. (+) indicates presence while (-) indicates absence of corresponding type of phytochemicals.

The beneficial effects of TCSBME were evident from the day of first measurement as progressive decrease in wound area was observed. On the 2<sup>nd</sup> day of wounding, the wound area shrinkage in TCSBME treated group was most when compared to the other groups. The wound area was decreasing constantly and predominantly in TCSBME group. The decline in wound area was twice more effective than the standard on 8<sup>th</sup> and 16<sup>th</sup> day. While the observations for TCSBAE were much similar to that of standard. The fastest epithelialization

Table 3. Results of Excision wound model.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 8</th>
<th>Day 16</th>
<th>Period of Epithelialization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>4.90±1.02</td>
<td>4.65±0.95</td>
<td>3.80±0.23</td>
<td>2.15±0.075</td>
<td>26±0.44</td>
</tr>
<tr>
<td>Standard</td>
<td>4.90±1.16&lt;sup&gt;**&lt;/sup&gt;</td>
<td>4.52±0.88&lt;sup&gt;**&lt;/sup&gt;</td>
<td>2.01±0.40&lt;sup&gt;**&lt;/sup&gt;</td>
<td>1.06±0.53&lt;sup&gt;**&lt;/sup&gt;</td>
<td>19±0.54&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>TCSBME 5% w/w</td>
<td>3.80±0.85&lt;sup&gt;**&lt;/sup&gt;</td>
<td>3.14±0.71&lt;sup&gt;**&lt;/sup&gt;</td>
<td>1.13±0.35&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.12±0.048&lt;sup&gt;**&lt;/sup&gt;</td>
<td>17±0.54&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>TCSBAE 5% w/w</td>
<td>4.67±0.94&lt;sup&gt;**&lt;/sup&gt;</td>
<td>4.40±0.68&lt;sup&gt;**&lt;/sup&gt;</td>
<td>2.46±0.47&lt;sup&gt;**&lt;/sup&gt;</td>
<td>1.13±0.062&lt;sup&gt;**&lt;/sup&gt;</td>
<td>19±0.33&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: Values in each column represent progressive change in wound area in mm as Mean ± Standard Error for 6 animals in each group, ns = not significant, * & ** indicate p < 0.05 & p < 0.01 respectively when compared to the control group.
Figure 1. Images of rats subjected to excision wound model on 1st and 19th Day. Note: A = negative control group B = TCSBAE group, C = TCSBME group Rats on 1st Day. D = standard group, E = TCSBAE Group and F = TCSBME Group Rats on 19th Day of the Experiment.

was recorded for TCSBME i.e. 17th day. While both standard and TCSBAE exhibited same period of epithelialization i.e. 19th day. Thus both TCSBME and TCSBAE (5% w/w) ointments promoted significant wound healing. The wound healing potential of TCSB-ME and TCSBAE can be attributed to the phytochemicals like anthraquinone glycosides, flavonoids and tannins present in it. An extensive literature is available that demonstrates the mechanism and positive role of above phytoconstituents in wound healing process.

Phytochemicals are receiving much attention as potential natural anti-oxidant in terms of their ability to act as both efficient radical scavengers and metal chelators (Nagulendran et al., 2007). A high correlation coefficient is reported between the phenolic content and anti-oxidant activities for various food commodities (Akond et al., 2010). Polyphenols can increase the activity of catalase and glutathione peroxidase that detoxify H$_2$O$_2$ by converting it to O$_2$ and H$_2$O (Khan et al., 1992; Oka et al., 2005). They are also known to stimulate wound healing (Sasidharan et al., 2010). Any drug that inhibits lipid peroxidation is believed to
increase the viability of collagen fibrils by increasing the strength of collagen fibers, preventing the cell damage and by promoting the DNA synthesis (Pradhan et al., 2009). Tannins are one of the most widely occurring groups of natural substances in different families of higher plants. These are secondary metabolites and chemically they contain a mixture of complex organic substances in which polyphenols are present. (Kokate et al., 2008). They exhibit potential anti-bacterial, anti-viral and anti-parasitic effects (Seeram et al., 2005). Tannins are found in a variety of herbal products used for wound healing. Their astringent and antimicrobial property is responsible for wound contraction and increased rate of epithelialization (Pradhan et al., 2009). Medicinal plants that are known and/or used for their wound-healing or antiinflammatory properties tend to have high tannin contents (Araújoa et al., 2008).

Flavonoids, a large group of natural products widely distributed in nature (Galicka et al., 2001) are documented to possess potent anti-oxidant and free radical scavenging effect, which is believed to be one of the most important components of wound healing (Shenoy et al., 2009). Bio-flavonoids are thought to benefit connective tissue by binding to elastin, preventing its degradation by elastases (Galicka et al., 2001). They reduce lipid peroxidation not only by preventing or slowing the onset of cell necrosis but also by improving vascularity (Pradhan et al., 2009). The high mobility of the electrons in the benzenoid nucleus of flavonoids accounts for both their anti-oxidant and free-radical scavenging properties (Havsteen, 2002). Many studies have shown that anti-microbial activities of plants can be attributed to their flavonoid content (Owoyele et al., 2008); hence, they are helpful in prevention of wound infection. Most of the delay in wound healing is due to insufficient or excessive fibroblast activity. Thus, inhibition of fibroblast growth by flavonoids such as apigenin could be beneficial for the treatment of any skin injury.

Flavonoids like rutin, naringin and quercetin protect DNA damage induced by ultraviolet radiation (Yeh et al., 2005). The chemical diversity, size, three-dimensional shape, and physical and biochemical properties of flavonoids allow them to interact with targets in different subcellular locations to influence biological activity in plants, animals and microbes (Buer et al., 2010). Saponins are known to promote wound healing process due to their anti-oxidant and anti-microbial activities (Sachin et al., 2009). Topical application of a saponin Asiaticoside, one of active constituents of Centella asiatica at a concentration of 0.2% for seven days to punch wounds in guinea pigs resulted in 56% increase in hydroxyproline, 57% increase in tensile strength, increased collagen content, and better epithelialization (MacKay and Miller, 2003). Triterpene saponins are also reported to posses-s immune-modulatory properties (Havsteen, 2002).

The present study reveals presence of secondary plant metabolites like alkaloids, anthra-quinone glycosides, flavonoids, saponins and tannins in TCSBME and TCSBAE. These phytoconstituents are known to promote the wound healing process due to their wide range of medicinal properties as discussed earlier. Hence, it is concluded that the wound healing potential of T. coriacea {Roxb.} Wight & Arn. stem bark extracts could be due to these phytochemicals and there exists a possibility of synergism between them. On the basis of findings, it can also be stated that TCSBME (5% w/w) ointment was most effective and exhibited most significant wound healing effect of all the positive treatments used in the experiment (TCSBAE and Povidone iodine, Betadine 5% w/w ointments). The earliest period of epithelialization observed was of group receiving TCSBME i.e., 17±0.54 days, while it was almost the
same (19 days) for groups receiving standard (Povidone iodine 5% w/w ointment) and other test (TCSBAE 5% w/w ointment). Early fall of scar & recovery of wound with premature development of hair in TCSBME treated group, indicates hair-growth promoting property. Further investigations are needed to isolate & characterize bioactive compounds and to understand the mechanism involved.

Acknowledgment

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Conflict of Interest

The authors have declared that there is no conflict of any competing interest.

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