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Wound Healing Activity of *Memecylon umbellatum* Burm

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Abstract: The present study was aimed to evaluate the wound healing activity of alcoholic leaf extract of *Memecylon umbellatum* Burm (Melastomataceae). On the basis of its literature references, this plant was selected for evaluation of its wound healing potential. An alcoholic leaf extract of *Memecylon umbellatum* Burm was examined for its wound healing activity in the form of ointment (0.5, 1.0 and 2% w/w) in two types of wound models in rats the excision and the incision wound model. The extract ointments showed significant response ($p<0.001$) in both the wound models as comparable to those of a standard drug nitrofurazone (NFZ) ointment (0.2% w/w) in terms of wound contracting ability, wound closure time and tensile strength.

Key words: *Memecylon umbellatum* Burm, wound healing, excision wound model, incision wound model, nitrofurazone, ethanolic leaf extract

INTRODUCTION

Wound may be defined as a loss of breaking of cellular and anatomic or functional continuity of living tissue (Yeo *et al*., 2000; Patil *et al*., 2001). Wound healing is a complex phenomenon involving a number of processes, including induction of an acute inflammatory process, regeneration of parenchymal and connective tissue cells, synthesis of Extra Cellular Matrix (ECM) proteins, remodeling of connective tissue and parenchymal components and acquisition of wound strength (Ramzi *et al*., 1994; Sunil *et al*., 1998).

Several nutritional factors required for wound repair may improve healing time and wound outcome. Vitamin A (Hunt, 1986), Vitamin C (Levine, 1986; Mazzotta, 1994), Vitamin E (Bartolomucci, 1939; Ehrlich *et al*., 1972), Thiamine (Vitamin B_1) (Alvarez and Gilbreath, 1982), Pantothenic acid (Vitamin B_5) (Aprahamian *et al*., 1985), Glucosamine sulfate and Chondroitin sulfate (Morrison and Murata, 1974), Bromelain (Tassman *et al*., 1965), trace elements such as Zinc (Pories *et al*., 1967), Copper (Rucker *et al*., 1998), Manganese (Tenaud *et al*., 1999; Pereira and Felcman, 1998) and Silicon (Carlisle, 1986; Leach, 1971) is required for crosslinking (and strengthening) of connective tissue, epithelial, collagen and bone formation, immune function and as a tissue antioxidant for proper wound healing.

A topical preparation of aescin (obtained from horse chestnut) (Guillame and Padioleau, 1994), Chamomile (Nasemann, 1975), Chaparral (Kay, 1996) and honey (topical application) (Forest, 1982) has been used topically to decrease inflammation and pain and promote healing of minor wounds. Adequate dietary protein is absolutely essential for proper wound healing and tissue levels of the amino acids arginine and glutamine may influence the protein synthesis in wound repair and immune

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function (Barbul *et al*., 1994). The botanical medicines *Centella asiatica* (Sunil *et al*., 1998), gotukola (Kay, 1996) and *Aloe vera* (Guillame and Padioleau, 1994; Nasemann, 1975) have been used for decades, both topically and internally, to enhance wound repair. The process of wound healing occurs in different phases such as coagulation, contraction, epithelization, granulation, collagenation and tissue remodeling (Pandarinathan *et al*., 1998). The extracts or formulations with wound healing potential may promote any one of the above process.

Memecylon umbellatum Burm (Family-Melastomataceae) commonly known as Iron wood tree (English), Alli and Puvai (Tamil) (The Wealth of India, 1998), is a large ornamental shrub or a small tree found mostly in the coastal regions of the Deccan peninsula, the eastern and southern parts of India and in the Andaman Islands (The Wealth of India, 1998). The leaves have been reported to possess astringent properties and are given to treat leucorrhea and gonorrhea (The Wealth of India, 1998; Lr. Colonel, 1993), a lotion prepared from the leaves is used to treat eye troubles (Sastri, 1962; The Wealth of India, 1998). The leaves are also reported to possess antiviral activity (The Wealth of India, 1998; Dhar *et al*., 1968). The leaves and the barks are applied to bruises (The Wealth of India, 1998). It contains wide variety of phytoconstituents which are useful in the treatment of different ailments and include umbelactone (4-hydroxymethyl-3-methyl-but-2-ane-4,7-olide), β-amyrin, sitosterol, its glucoside, tartaric, malic, oleanolic and ursolic acid (Asolklar *et al*., 1956; Ram and Mehrotra, 1993).

Despite recent advances in antimicrobial chemotherapy several types of superficial wounds still show resistance to routine wound treatment and are prone to infections (Hurt, 1970). Antibiotics are of little help in such cases because these skin ulcers often contain microorganisms that are drug resistant. Moreover in many cases antibiotics produce adverse side effects and allergy (Chirife *et al*., 1982). In traditional systems of medicine, various plants have been used as an excellent adjuvant for accelerating wound healing activity.

A survey of literature revealed that no systematic approach has been made to study the wound healing activity of this plant. Hence the present study was carried out to evaluate the wound healing activity of alcoholic leaf extract of *Memecylon umbellatum* Burm in different models of experimental wounds.

MATERIALS AND METHODS

Plant Material

Fresh leaves of *Memecylon umbellatum* Burm was collected from Pacchaimalai hills, Tiruchirappalli district, Tamilnadu, India during the months of March-Apirl 2004. The identity of the leaves has been confirmed by using all official monographic specifications. Leaves were dried under shade, pulverized by an electric blender and passed through 40 mesh sieve. It was stored in an air tight pearlpet® container, away from sunlight at room temperature and used for the study.

Preparation of Extract

The powdered leaves of *Memecylon umbellatum* Burm (500 g) was extracted with ethanol (95%) in a soxhlet extractor. The extract was evaporated to dryness at 60°C under reduced pressure in a rotary evaporator. The residue thus obtained constituted 30% of the original material.

The different concentrations (0.5, 1 and 2% w/w) of extract ointments were prepared on simple ointment base BP (British Pharmacopoeia, 1993). The extract ointments (0.5, 1 and 2% w/w) at a quantity of 0.5 g were applied once daily to treat different groups of animals. The simple ointment base and 0.2% w/w nitrofurazone (NFZ) ointment were applied in the same quantity to serve as control and standard, respectively.

Animal Experimentation

Male *albino rats* of *Wistar strain*, weighing about 150-200 g was used for the experiment. They were fed with standard pellet diet (M/s. Hindustan Lever Limited, Bangalore, India) and water was given *ad libitum*. They were housed in poly propylene cages under standard conditions (12/12 h light/dark cycle; 25±3°C on 60-70% RH). The protocol for animal experimentation was approved by the Institutional Animal Ethical Committee (IAEC) with Reg. No. 418/a/01/CPCSEA and conducted according to the guidelines of Indian National Academy for the use and care of experimental animals. The animals were acclimatized for one week under laboratory conditions before performing the test. Each animal was used only once.

Test Animals

The animals were divided into five groups of six rats each as follows: Group I- rats were treated with simple ointment base (control), Group II-rats treated with reference standard (0.2% w/w NFZ ointment), Group III, IV and V-rats were treated with 0.5, 1 and 2% w/w of extract ointments, respectively.

Effect on Excision Wounds

In the excision wound model (Saha *et al*., 1997; Puratchikody *et al*., 2006), the full thickness excision wounds were made on the rats by removing a 500 mm^2 piece of skin from the shaven backs after anaesthetized with anesthetic ether by the open mask method. After skin incision, the wound was left open to the environment. Male *albino rats* of *Wistar strain* (150-200 g) were used in the study and worked up as above. The groups were treated in the same manner as mentioned in the animal experimentation or test animals. The extract ointment (0.5, 1 and 2% w/w) at a quantity of 0.5 g were applied once daily to treat different groups of animals, while simple ointment base and 0.2%w/w nitrofurazone ointment were applied in the same quantity to serve as control and standard respectively. Wound healing potential was monitored by wound contraction and wound closure time.

Wound contraction was calculated as reduction in wound area (Table 1). The progressive changes in wound area were monitored planimetrically by tracing the wound margin on graph paper on wounding day followed by sixth, twelfth and eighteenth day (Tenaud *et al*., 1999; Leach, 1971; Udupa *et al*., 1994; Morton and Malone, 1972; Bairy and Rao, 1993; Saha *et al*., 1997).

Effect on Incision Wounds

For incision wound model (Udupa *et al*., 1994) five groups with six animals in each group were anaesthetized with anesthetic ether and 6 cm long paravertebral long incisions were made through the skin and cutaneous muscle at a distance of about 1.5 cm from the midline on each side of the depilated back of the rats. After the incision was made, the parted skin was kept together and stitched at 0.5 cm intervals using surgical thread (No. 000) and a curved needle (No. 11). The threads on both wound edges were tightened for good closure of the wound. All the groups were treated in the same manner as mentioned in the case of excision wound model. Extract ointments, simple ointment base (control) and standard drug were applied once daily for 9 days; when the wounds were cured thoroughly the sutures were removed on day 9 and the tensile strength of the healed wound (Ehrlich and Hunt, 1969) was measured on day 10 by continuous and constant water flow technique by the method of Lee (Bairy *et al*., 1995; Lee, 1968).

Statistical Analysis

The results were expressed as mean±SEM (Standard Error of Means) and statistical significance was evaluated by using student's t-test vs control group. $p<0.001$ implies significance (Armitage, 1971).

RESULTS AND DISCUSSION

The effect of extract ointments (0.5, 1 and 2 %w/w), NFZ ointment (standard) and simple ointment base (control) in the excision wound model and in the incision wound model were assessed by measuring the wound area and tensile strength, respectively. The data including wound area (mm^2) and tensile strength of healed wound was furnished in Table 1. The present investigation revealed that the test extract in varying concentration in the ointment base were capable of producing significant wound healing activity on both wound models. The entire test extract ointments used in excision wound model showed significant increase in rate of wound contraction on the days 12 and 18 (Fig. 1). The results in Table 1 indicate that, out of the three extract ointments used in the experiment, ointment prepared with 2% w/w of alcoholic extract of *Memecylon umbellatum* has been found to have relatively more wound healing activity with 100% wound closure on day 18 is as to the standard (NFZ ointment, 0.2% w/w). The other two extract ointments (0.5 and 1% w/w) didn't show much difference in their effects on wound closure (Table 1).

Table 1: Effect of alcoholic extract of *Memecylon umbellatum* Burm ointments on excision and incision wound

	Excision wound model wound area in (mm^2)±SEM post wounding days				Incision wound model
Treatments	0 day	6th day	12th day	18th day	Tensile strength (g±SEM)
Simple ointment B.P.	538.25±1.500	288.50±5.908	279.20±3.096	168.00±1.090	405±13.4
Nitrofurazone ointment (0.2%w/w)	515.70±4.055	285.50±8.808	83.00±1.416	0.50±0.5	548±11.2
Extract ointment (0.5%w/w)	524.74±4.950	325.00±5.592	87.50±1.510*	9.78±0.853*	532±10.1
Extract ointment (1.0 %w/w)	525.50±8.720	306.50±6.305	85.00±2.860*	6.76±0.750*	536±10.7*
Extract ointment (2.0 %w/w)	527.75±5.261	287.00±3.104	71.20±1.315*	0.00±0.00*	543±11.2

Test drugs: significance from standard *p<0.001 (n = 6), p vs control by student's t-test

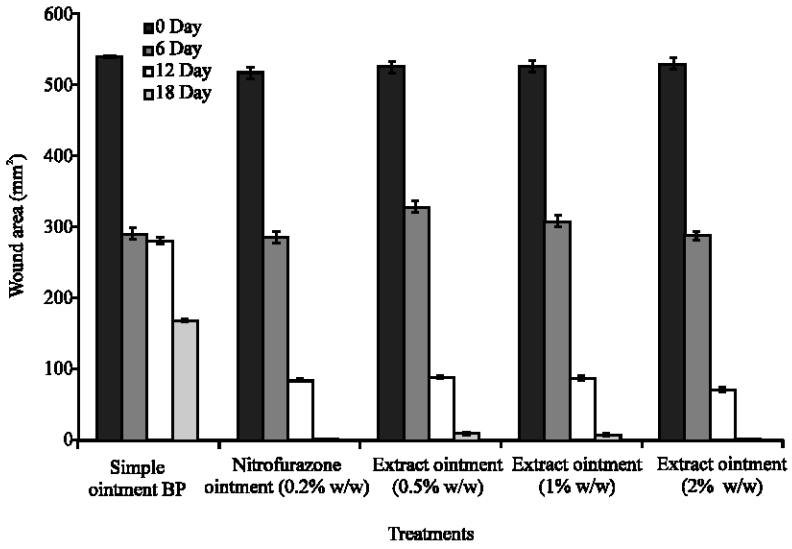

Fig. 1: Effect of alcohol extract of *Memecylon umbellatum* Burm ointments on excision wound model

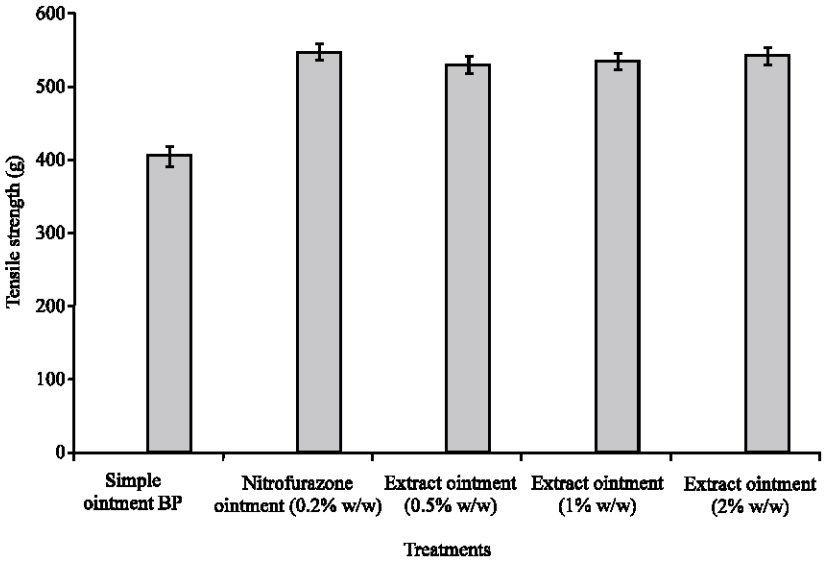

Fig. 2: Effect of alchol extract of *Memecylon umbellatum* Burm ointments on excision and incision wound

In the incision wound studies, there was a significant increase in tensile strength on day 10 due to treatment with either the extract ointments or the standard nitrofurazone when compared to control. The effect produced by the nitrofurazone ointment (0.2% w/w) application was found to be same (Fig. 2) as that obtained with the application of the extract ointment (2% w/w).

In the incision wound model, increase in tensile strength of treated wounds may be due to increase in collagen formation/unit area and stabilization of the fibres (Mukherjee *et al*., 2000; Taranalli and Kuppast, 1996). Improved collagenation was observed in extract ointment treated groups in a dose dependent fashion as compared to groups treated with Simple ointment base BP. Ursolic acid was the only component which may be responsible for collagen synthesis stimulation.
The process of wound healing occurs in four phases:

- Coagulation, which prevents blood loss,
- Inflammation and debridement of wound,
- Repair, including cellular proliferation and
- Tissue remodeling and collagen formation (Evans, 1980).

Any agent who accelerates the above process is a promoter of wound healing. Plant products have been seen to possess good therapeutic potential, due to the presence of active terpenes such as ursolic acid, α-amyrin and β-sitosterol.

Memecylon umbellatum Burm contains ursolic acid (3α-hydroxy-uro-12-en-28-oic acid) an isomer of oleanolic acid, is a triterpenoid compound exhibits broad spectrum of pharmacological properties such as hepatoprotective (against carbon tetrachloride, D-galactosamine induced liver injury and acetaminophen induced cholestasis), analgesia, anti-inflammatory, antiviral, anti-microbial, anti-choletation, choleretic, anti-mutagenic, anti-hyperlipidemic, anti-tumour activity, anti-ulcer, anti-carcinogenic, anti-arthritic and antifertility activity (Tiwari *et al*., 1998).

Out of the three extract ointments used, extract ointments (2% w/w) appears to be the best in promoting the wound healing. The results are in accordance with the report of Maquart *et al*. (1990) who reported that triterpenes of *Memecylon umbellatum* Burm stimulated collagen synthesis in

cultured fibroblasts revealed a positive healing profile and increase in tensile strength (Rosen *et al*., 1967). The wound healing property of *Memecylon umbellatum* appears to be due to the presence of active principles, which accelerates the healing process and confers breaking strength to the healed wound. On the basis of the results obtained in the present investigation, it is possible to conclude that the ointment of the extract of *Memecylon umbellatum* Burm has significant wound healing activity at all the doses tested.

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