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Research Article

Wound Healing Effect of Furfural and Pentadecanal from *Lagerstroemia speciosa* (L.) Pers Acetone Flower extracts against *Haemadipsa sylvestris* Bite

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ABSTRACT

Health professionals working in rural areas where leech infestation is common should be aware that people are at risk for leech bites. The bite develops infected and wounds or ulcers may cause a reaction to an allergen. The experimental plant *Lagerstroemia speciosa* is commonly called Queen crape myrtle. Medicinal plants as effective and affordable treatments for cutaneous wounds. The objective of the present study was to investigate the wound healing effect of acetone flower extract of *L. speciosa* against *Haemadipsa sylvestris* bite. Group I control. They didn't receive any drug treatment. Group II and III received commercial ointment, betadine and herbal ointment, respectively. Group IV and V are the topical application of LAFE 1 and 2% formulated ointments. The results revealed that the topically applied herbal ointment-treated groups had a rate of wound contraction of 94.4%. The topically applied LAFE 1% and LAFE 2% treated groups had rates of wound contraction of 94.5 and 95.3% (p <0.05), respectively at day 21. The present research finds the LAFE1% treated group has equal contraction to the herbal ointment applied group and the LAFE2% treated group has the highest contraction rate compared to the control. The study revealed that the acetone flower extract of *L. speciosa* was capable of promoting wound healing and functional recovery in a dose-dependent way.

Keywords: Leech infestation, Flower extract of *Lagerstroemia speciosa*, *Haemadipsa sylvestris* bite, Betadine, Herbal ointment, Wound contraction.

INTRODUCTION

A wound is defined as an opening or breaking in the integrity of the skin that causes disruption of the anatomical and functional integrity of living tissues.[1] The wound-healing process has the purpose of recovering the integrity of the damaged tissue and the regeneration of the epithelium that was lost, and it is a dynamic and complex process that globally occurs in four overlapping steps: hemostasis, inflammation, tissue proliferation, and regeneration.[2]

In India, drugs of herbal origin have been used underlying the medical culture of India. Both folk traditions as well as codified knowledge systems are a deep understanding of the medicinal value of plants.[3] The Indian systems of medicine have been a part of the culture and tradition of India down the centuries. *Lagerstroemia speciosa* comes under the family Lythraceae is known as the "Pride of India". It was native to Asia-tropical and subtropical regions. It is commonly called Poomaruthu in Tamil, Manimaruthu in Malayalam, and Jarul or Banaba in many countries. *L. speciosa* is a common ornamental tree planted along roadsides, gardens and parks. This species has been traditionally used in folk medicine as a remedy for illnesses and ailments.[4]

Leeches are bloodthirsty, segmented, parasitic or predatory hermaphroditic annelids, which belong to the subclass Hirudinea. Haemadipsa is a genus of leeches with members commonly known as jawed land leeches. These annelids are known from subtropical and tropical regions around the Indian and Pacific Oceans. *Haemadipsa sylvestris* is a species of blood-feeding leech recorded from India, Nepal, Sri Lanka, Myanmar, Malaysia, and Indonesia. A species of freshwater crab, *Himalayapotamon atkinsonianum*, has been recorded as a host.[5] Members of the genus feed on blood. They are troublesome to humans and animals especially because their bites result in prolonged bleeding. Leeches can be very discriminating in their feeding patterns, preferring blood from certain species.[6]

The aim of the study is to control the wound caused by the parasitic worm *H. sylvestris* bite by the bioactive compound furfural and pentadecanal from the medicinal plant *L. speciosa* acetone flower extract.

MATERIALS AND METHODS

Collection and Authentication of Experimental Sample

The flowers of *Lagerstroemia speciosa* were collected from PG Girls hostel, Government Arts College (Autonomous), Coimbatore District, Tamil Nadu, India. The *L. speciosa* were identified and authenticated by the Botanical Survey of India, Tamil Nadu Agriculture University, Coimbatore-03 (No: BSI/SRC/5/23/2020/

Tech/57) and the voucher specimens were kept in the Department of Zoology, Government Arts College, Coimbatore-18.

Flower Extract Preparation

The collected flower samples were to clean the dust and any other particles stuck to them. The samples were then kept under the shade at room temperature (27 ± 2°C) for about two weeks till they dried completely. They were finely powdered using an electric blender. The powdered material (100 g) was soaked in acetone (1000 mL) in an airtight, wide-mouthed bottle and kept for four days with periodic shaking. After that, the extract was filtered using Whatman No. 1 filter paper and kept in a plastic tray to dry at room temperature.[7]

Phytochemical Analysis

Qualitative phytochemical analysis of the acetone flower extracts was carried out according to the methodology of Horborne (1984)[9] and Trease and Evans (1989)[10] (Table 1).

Ointment preparation

The fusion method was employed in the preparation of ointments. The required quantity of ointment base weighed and melted at a temperature of about 70°C in a water bath. The designated quantity of the extract was respectively added to the melted base at 40°C and the mix stirred gently and continuously until a homogenous dispersion was obtained. Herbal ointments containing 1 and 2% w/w of the flower extract were prepared. For each fraction, two concentrations of 1 and 2% w/w were formulated in sterile soft white paraffin. Immediately after preparation, the ointments were aseptically transferred into sterile cream tubes and sealed.[11]

Experimental Animals

Albino rats

Wistar strain of healthy adult male albino rats (150–200 g) were used for this experiment. The animals were housed in standard metal cages in a room maintained at 22 ± 1°C with an alternating 12 hours light-dark cycle. Food and water were available *ad libitum*.

Collection of albino rats and ethical approval

The Albino rats were collected from the animal house KMCH College of Pharmacy. The Institutional Animal Ethics Committee, KMCH College of Pharmacy, and Coimbatore approved all the experimental procedures used in these studies. (Approval No: KMCRET/ ReRc/ Ph.D. /30/ 2021).

Collection of leech

Leeches are collected from the Estates of O'Valley, Gudalur Taluk, with latitude and longitude of 11°27'19''N and 76°28'79''E. O'Valley or Ouchterlony Valley is a Town panchayat in the Gudalur Taluk of the Nilgiris district in the Indian state of Tamil Nadu. The place being located between Mudumalai National Park and New Amarambalam Reserved Forest is famous for its flora and fauna. The moist and humid climate causes heavy infestation of land leeches in jungles, hill slopes and pathways. Leeches remain inactive during hot weather but become active during light drizzle.[11]

Maintenance of Leech

In this study, the number of experimental animals is around 25. *H. sylvestris* leeches, with uniform sizes, are the largest and most robust species of the Indian land leeches. Leeches were gathered from their natural environment, specifically from decaying leaves and under rocks. Leeches were manually picked and transferred into a plastic container and kept wet by adding a small amount of water and moist soil; the collected leeches were transported to the laboratory.

Identification of Leech

The identification of these land leeches was made with the help by using "The Leeches of India: A Handbook" by Chandra M (1991).[12]

Skin Irritation Study

This test was performed on albino rats weighing between 150 to 200 g. The animals are divided into five groups, with each batch containing five animals. The dorsal hairs at the back of the rats were removed one day before the commencement of the study and kept individually in cages to avoid contact with the other rats. The experimental wound ointment was applied topically to groups IV and V, while the other two ointments were used to compare skin irritation in rats. At the end of the study, animal skin was evaluated for its irritancy and sensitization effect. The animals were observed for seven days for any signs of edema and erythema.[13]

Experimental Design

For the excision wound model, five groups of healthy adult rats (n = 5) were used.

Group I – Control group did not receive any cream or drug treatment
Group II – Animals treatment with Betadine
Group III – Animals treatment with Herbal ointment
Group IV – Animals treatment with LAFE 1% w/w
Group V – Animals treatment with LAFE 2% w/w

Excision Wound Model

Slightly modified, an excision wound was created according to the Morton and Malone method (1972).[14] Animals were anesthetized. The dorsal fur of the animals was shaved with an electric clipper. After that, the exposed area the experimental animal allows to bite. Finally, the excision wound area was marked and calculated. Hemostasis was achieved by blotting the wound with a cotton swab soaked in normal saline. All the procedures were performed under aseptic conditions.

The treatment schedule was twice daily with topical application of the formulated ointment as well as the standard drug ointment, while the control group was dressed with an ointment base containing the same quantity of petroleum gel. Sterile cotton swabs were used for the uniform application of ointments.

Wound Contraction Calculation

Wounds were traced on 1 mm^2 graph paper on the day of wounding and subsequently on alternate days until healing was complete. Changes in wound area were calculated, giving an indication of the rate of wound contraction.

$$\text{Wound contraction (\%)} = [A_o\text{-}A_t]/A_o*100.$$

Where, A_0 – the initial wound area, A_t – the wound area after time interval "t."

The length of newly generated epithelium across the surface of the wound was determined as the sum of the new epidermis growing from the right and left margins of the wound.[15]

Statistical Analysis

All the experimental values are expressed as the mean ± SEM. Statistical significance (p) by one way ANOVA followed by Dunnett's test. $^{**}p < 0.05$ was calculated by comparing treated group with the control group.

RESULTS

Result 1: Phytochemical analysis of Acetone Flower extract of *Lagerstroemia speciosa*

The qualitative phytochemical analysis of acetone flower extract revealed the presence of the following bioactive compounds: Alkaloids, flavonoids, saponins, phenols, tannins, protein and amino acids, reducing sugar, steroids, glycosides, phytosterols, coumarins, quinones (Table 1).

Result 2: Skin irritation test of *Lagerstroemia speciosa* Acetone Flower extract of formulated ointments

The experimental plant *L. speciosa* acetone flower extract is used for the preparation of wound ointments (1% and 2% LAFE) that were applied topically on the fourth and fifth groups; there was no sign of edema or erythema noted from the observation.

Table 1: Phytochemical analysis of acetone flower extracts *Lagerstroemia speciosa*

S. No	*Phytoconstituents*	*LAFE*
1	Alkaloids	+++
2	Flavonoids	+++
3	Saponins	+++
4	Phenols	+++
5	Tannins	+++
6	Protein and Amino acids	+++
7	Reducing sugar	+++
8	Steroids	+
9	Glycosides	++
10	Phytosterols	++

'+' indicates the presence of Phytoconstituents
'++' indicates the Phytoconstituents present in a moderate level
'+++' indicates the Phytoconstituents present abundantly

Result 3: Wound healing activity of Acetone Flower extract of *Lagerstroemia speciosa* on *Hirudo sylvestris* bite wounds

The wound contraction rate determined the wound healing activity. The excision wound model studies have indicated that the rate of wound contraction in the control group is 92.3%. The Betadine standards-treated rats showed an increase in wound contraction by 100%. The topically applied herbal ointment-treated groups had a rate of wound contraction of 94.4%. The topically applied LAFE 1% and LAFE 2% treated groups had rates of wound contraction of 94.5 and 95.3% ($p < 0.05$), respectively, at day 21 (Table 2 and Fig. 1).

DISCUSSION

The rate of wound contraction determined the healing activity. The rate of wound contraction depends mainly on the size of the wound. It is known that collagen accumulation is the sum of collagen synthesis and destruction, which both occur simultaneously during the wound-healing process.[16] Phenolic compound tannins and triterpenoids[17] are known bioactive compounds to promote the wound healing process mainly due to their astringent and antimicrobial properties, which seem to be responsible for wound contraction and increased rate of epithelization.

The control group shows normal healing because there are underlying clotting abnormalities, hemostasis happens shortly after the original injury. By secreting clotting factors that constrict blood vessels, platelets seal off the injured blood vessels. Experimental group IV shows an equal recovery rate compared to that of group III, and group V shows a higher recovery rate compared to control and

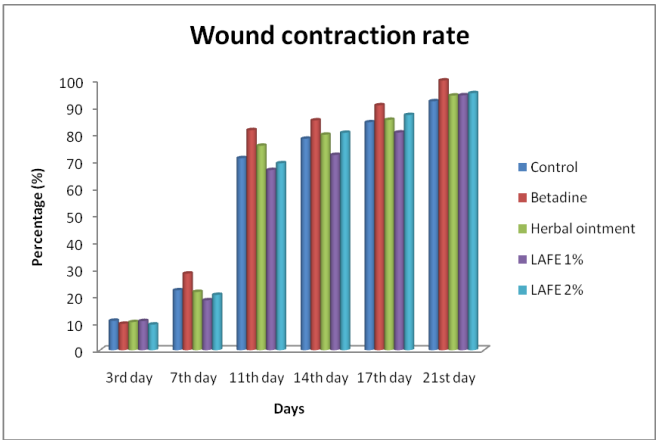

Fig. 1: Wound contraction activity of acetone flower extract of *Lagerstroemia speciosa* on *Haemadipsa sylvestris* bite

Table 2: Wound contraction rate of acetone flower extract of *Lagerstroemia speciosa*

Groups	*Control*	*Betadine*	*Herbal ointment*	*LAFE 1%*	*LAFE 2%*
3rd day	10.9 ± 6.21	9.8 ± 1.24	10.4 ± 5.33	10.8 ± 5.04**	9.5 ± 3.25
7th day	22.2 ± 2.35	28.4 ± 6.17	21.6 ± 0.71**	18.5 ± 4.77	20.5 ± 2.76
11th day	71.2 ± 2.84	81.6 ± 3.98	75.8 ± 4.70**	66.8 ± 4.27	69.3 ± 10.89
14th day	78.4 ± 4.30	85.2 ± 4.91	79.9 ± 8.3	72.4 ± 9.84	80.6 ± 4.88**
17th day	84.5 ± 1.58	90.8 ± 3.12	85.4 ± 2.95	80.7 ± 9.44	87.2 ± 4.72**
21st day	92.3 ± 1.78	100 ± 0	94.4 ± 1.96	94.5 ± 1.16	95.3 ± 1.39**

Values are expressed as the mean ± SEM. Statistical significance $^{**}p < 0.05$ by one way of ANOVA followed by Dunnett's test.

lower to standard group. From the result, the study showed that the acetone flower extract of the *L. speciosa* phytocompounds Furfural and Pentadecanal was capable of promoting wound healing and functional recovery in a dose-dependent way. The phytochemical constituent may be responsible for the highest rate of wound contraction. This study supports *L. speciosa* potential as an ethno-medicinal remedy.

CONCLUSION

India is the second largest producer of tea in the world. Of this, 25% is contributed by Munnar and Nilgiris tea gardens. One of the major problems faced by the labors is worm infestations. The workers do not have proper connectivity to health centers because they are located in remote areas. In tropical regions, leech bites on the skin are usual. However, serious infections of leech bite injury are uncommon. If the infection occurs, it causes abnormal effects and may even be fatal. *Haemadipsa sylvestris* is a species that bites deeply and causes extensive bleeding from the wound after they are detached. The deep-rooted bleeding after a leech bite is because of the factors in the saliva left in the bite by the leech, which contains hirudin, hyaluronidase, histamine-like vasodilators and calin, a platelet aggregation inhibition.

Leech bites are generally alarming rather than dangerous, though a small percentage of people have severe allergic or anaphylactic reactions and require urgent medical care. It will create symptoms like red spots or an itchy rash all over the body, swelling, dizziness, and difficulty breathing. The leech bite is somewhat like an excision type of wound. The formulated ointments were topically applied to the leech bite wound. The bioactive components in the formulated ointments enhance the healing. In the present investigation, acetone flower extract of *L. speciosa* supports the healing of leech bites to promote wound contraction.

AUTHORS' CONTRIBUTIONS

Author 1* designed the study, wrote the protocol, and wrote the first draft of the manuscript;

Author 1 did the experimental work and performed the statistical analysis;

Author 2 managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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