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Histopathological Assessment of Excision Wound Healing Activities of *Lagerstroemia speciosa* Ethanolic Extracts of Leaf, Flower, and Seed in Tissue Sampling Biopsy of Male Albino Rats

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Abstract

Histological techniques are used to produce high-quality, thin tissue slices for evaluating abnormal animal tissue modifications. *Lagerstroemia speciosa* ethanolic extracts from leaf, flower, and seeds are evaluated for excision wound healing properties. The objective of the study is to analyze two methods: one is an animal model, and the other is evaluating tissue progression. For histological analysis, animals can be euthanized and biopsies collected, processed, and examined for epithelial gap, granulation bed characteristics, and collagen organization. Results show increased neovascularization, re-epithelialization, and collage in all groups' wound histology, except for the control group.

***Key words*:** *Lagerstroemia speciosa*, Biopsies, Tissue progression, Wound healing Ethanolic extracts

Wounds are breaks in living tissue's cellular, anatomical, and functional continuity caused by physical, chemical, thermal, microbiological, or immunological assaults. They can cause disruptions in normal tissue structure and function [1]. Techniques like morphology, cytology, and molecular biology assess wound life. Histopathology of wounds aids in diagnosis, tracking healing progress, understanding non-healing wound pathophysiology, and assessing morphological changes. The border of the wound is the best location for a biopsy, allowing comparison between ulcerated area and surrounding skin [2-3]. Medicinal plants possess medicinal properties, known as "God's signature," and are crucial to human life. *Lagerstroemia speciosa*, a deciduous tree in the Lythraceae family, is a popular choice in tropical and subtropical regions [4-5]. Excisional wounds are one of the most commonly used wound healing models [6]. This model allows the investigation of hemorrhage, inflammation, granulation tissue formation, epithelialization, angiogenesis and remodeling. The aim of the study is to analyze the excision wound activities of *L. speciosa* ethanolic extracts of leaf, flower, and seed in tissue sampling biopsy of Male albino rats.

MATERIALS AND METHODS

Collection and authentication of plant samples

The leaves, flowers and seeds of *L. speciosa* were collected from PG Girls Hostel, Government Arts College, Coimbatore District, Tamil Nadu, India. The *L. speciosa* were identified and authenticated at the Botanical Survey of India, Coimbatore - 03 (No. BSI/ SRC/ 5/ 23/ 2020/ Tech/ 51) and the voucher specimens was kept in Department of Zoology, Government Arts College (Autonomous), Coimbatore-18.

Preparation of plant extracts

The collected *L. speciosa* samples were thoroughly examined for any indications of illness or infection in order to isolate clean samples for the experiment. After that, the samples were kept in the shade for approximately two weeks at room temperature (27°C) until they were completely dry. The dried samples were processed into a powder using a mixer grinder. After that, 100g of the powder samples were soaked in 1000ml of ethanol solvent and kept in an airtight container for 4days while being stirred occasionally. The extract was then filtered through Whatman No. 1 filter paper, and left to air dry in petri dishes at room temperature [7].

Phytochemical analysis

Qualitative phytochemical analysis of the ethanolic leaf, flower and seed extracts of *L. speciosa* was followed by the methodology of Horborne [8] and Trease and Evans [9].

Drugs

The preparation of herbal ointment (1% w/w and 2% w/w of LELE, LEFE and LESE) was described in ointment preparation. The ethanolic leaf, flower and seed extracts (500mg) was uniformly suspended in 1% carboxymethyl cellulose (CMC) dissolved in water and administered orally (p.o). Povidone-iodine and Neomycin, commercially available ointments were purchased from the pharmacy.

Experimental design

The efficacy of *L. speciosa* ethanolic extracts to speed up healing wounds and act as antioxidants were examined.

Male albino Wister rats were provided with excision wounds that were then treated for 21 days with *L. speciosa* ethanolic extract. Povidone-iodine and Neomycin ointments

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were used as well as standard medicines for wound healing. The excision wound model studies showed that control rats experienced 84.5% to 92.3% wound contraction from day 17 to 21, while complete epithelization and healing occurred on day 24. Povidone-iodine and Neomycin standards treated rats showed 93.6% to 100% and 91.2% to 100% wound contraction, respectively.

Tissue samples

The experimental animals can either be euthanized and biopsies can be collected, processed, and examined for the epithelial gap, granulation bed characteristics and collagen organization. The materials and techniques necessary to use this model are relatively simple and practical. The wound bed can be easily accessed to apply topical agents and investigate their effects on the repair process [10-14].

Histopathological studies

Excised granulation tissue from the 3rd day and a specimen sample of tissue were isolated from the healed skin of each group of rat skin on the 21st day and fixed in 10% formalin before histological processing. Sections were made with hematoxylin and Eosin (H and E) staining, and assessment for histological features was observed under a light microscope.

Preservation of tissue biopsy

To preserve tissue samples' integrity without disrupting their cellular structure, they should be placed in fixatives like 10% buffered formaldehyde and stored at 80°C. Hematological processing stages include embedding, sectioning, and staining, with hematoxylin and eosin (H&E) being the most commonly used. Trichromes, immunohistochemical markers, and special stains are used to highlight invisible or less evident tissue components.

Collection of tissue sample

In addition to normal tissue, tissues exhibiting gross morbid alterations were obtained in thin pieces that ranged in thickness from 3 to 5 mm.

Fixation

Keep the tissue in the fixative at room temperature for 24 to 48 hours. Common fixatives: 10% formalin. The fixation served vital purposes: it served to harden the tissues by coagulating the cell protein, prevent autolysis, preserve the structure of the tissue, and prevent shrinkage.

Haematoxylin and eosin method of staining

Deparaffinize the section with xylol for 5 to 10 minutes, and then remove the xylol with absolute alcohol. Then I cleaned the section in tap water, stained it with haematoxylin for 3 to 4 minutes, and again cleaned it in tap water. Allow the sections to sit in tap water for a few minutes, then counterstain with 0.5% eosin until the section appears light pink (15–30 seconds), and then wash in tap water. Alcohol-based blotting, dehydration, and xylol clearing (15–30 seconds), mounted on a Canada balsam or DPX Mutants and kept the slide dry and removed air bubbles.

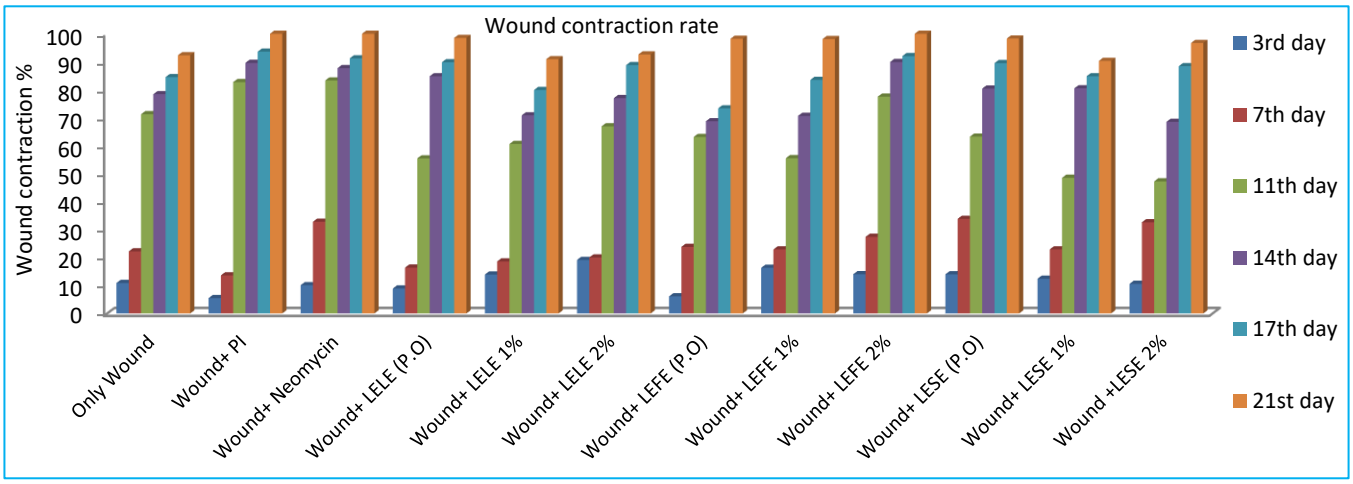


Fig 1 Wound contraction rate of ethanolic leaf, flower, and seed extracts of *Lagerstroemia speciosa*

Fig 2 Excision wound creation in experimental animal

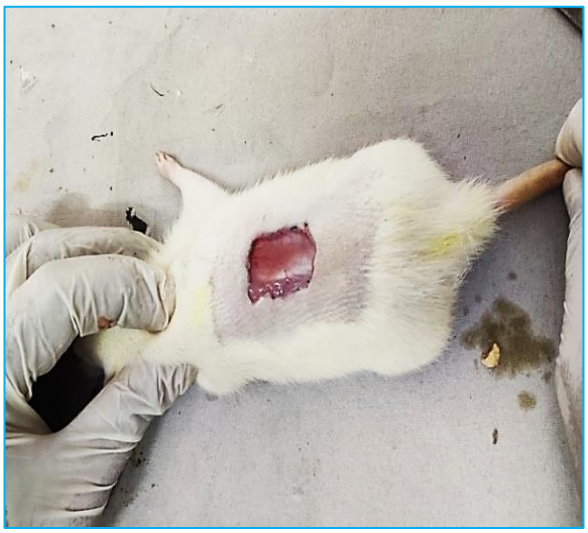


Fig 3 Wound area in excision wound created experimental animal

RESULTS AND DISCUSSION

As a result of influencing several stages of the healing process, the ointment made from the leaf, flower, and seed of *Lagerstroemia speciosa* significantly promoted healing in rats' infected wounds. The amounts of fibroblasts, fibrocytes, and vascular proliferation in the three groups in the evaluations after 21 days were not significantly different according to the histological analysis of the samples. In compared to the control group, there was a drop in collagen concentration on day three and a rise on day twenty-one.

Unlike human skin, which only has the panniculus carnosus layer in the platysma of the neck, rodent skin has this layer, which causes an injury to heal quickly. In contrast, human wounds recover through the production of granulation tissue and re-epithelialization [15], which are crucial distinctions to consider when determining the translational significance of rodent studies.

For instance, the thicker dermis of male skin makes it 40% stronger than that of female skin, which also has a thicker epidermis and subcutaneous layer. Rats are better for investigating wound healing due to their thinner skin and faster healing time. Mice's wounds are smaller and heal faster, but they can be used in small sample sizes for analysis. These models help understand skin biology and pathology [12].

Ethanol is used for quality control in plant identification due to bioactive components like tannins, flavonoids, and phenolic compounds. These compounds have homeostatic effects and promote wound healing. Therapeutic compounds like antioxidants, antimicrobials, anti-inflammatory drugs, and re-epithelialization agents are present in plant extracts like *Lagerstroemia speciosa* [16].

Polyphenols, including tannins and flavonoids, are natural antioxidants found in *Lagerstroemia speciosa* plants [17]. The experimental plant's ethanol leaf extract contains higher total protein content than standard groups. *Lagerstroemia speciosa* seeds have anti-inflammatory and anti-allergic properties, including furfural, which suppresses wound inflammation. The presence of plant hormones, including cytokinins, auxins, terpenes, carotenoids, and ethylene derivatives, suggests the plant's potential in treating infectious diseases. Medicinal plants and active compounds reduce inflammation, while keratinocytes and macrophages play crucial roles in wound healing and phagocytosis [18]. The study supports the pharmacological use of *Lagerstroemia speciosa* as a medicinal plant for wound healing, showing 84.5% to 92.3% contraction from days 17-21. Flavonoids in the plant can enhance tissue remodeling and act as proangiogenic agents. Further research is needed to identify additional mechanisms.

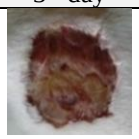
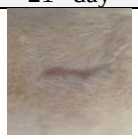
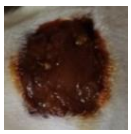
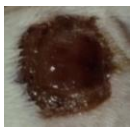
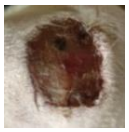
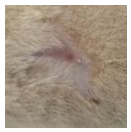
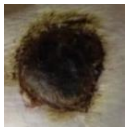
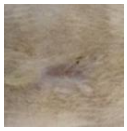
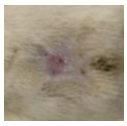
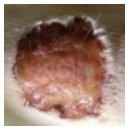
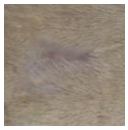
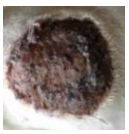
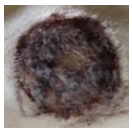
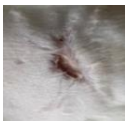
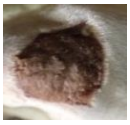
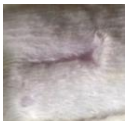
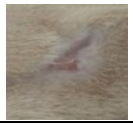
CONCLUSION

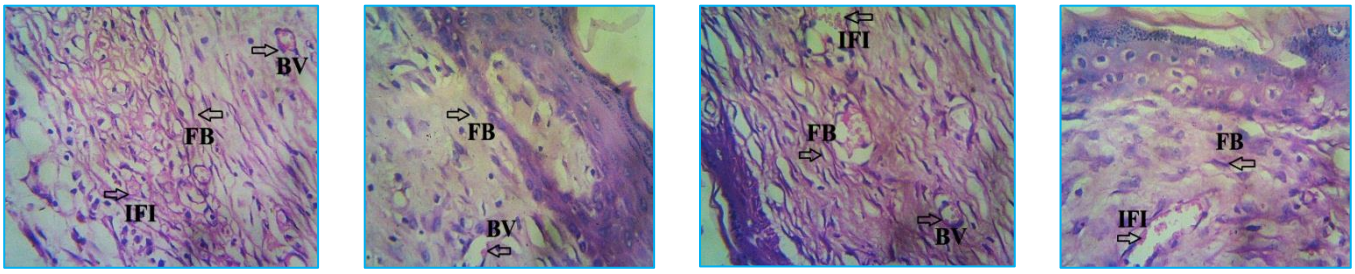
Wound healing involves three phases: inflammation, proliferation, and maturation. Wound healing is a complex series of reactions and interactions among cells and "mediators." The study assessed the ethanolic extract of *Lagerstroemia speciosa's* ability to heal wounds using an animal model. Results showed that oral administration and applied topically, the *L. speciosa* ethanol extract promoted faster wound healing than oral treatment and control groups, suggesting its potential as a wound healing agent.

Ethical approval

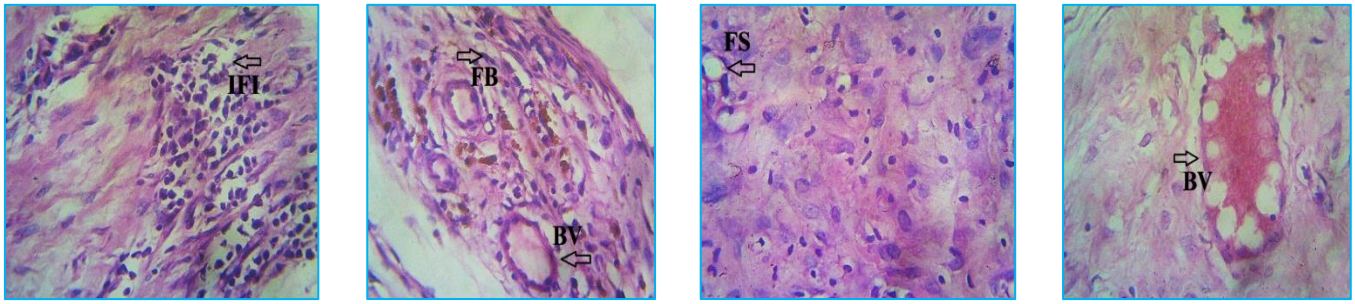
Animal experiments were performed at KMCH College of Pharmacy, Coimbatore (Approval No: KMCRET/ReRc/Ph.D/23/2021), Tamil Nadu, India. All the procedures were approved from the Institutional Animal Ethics Committee.

Fig 4 Shows wound area for histological analysis

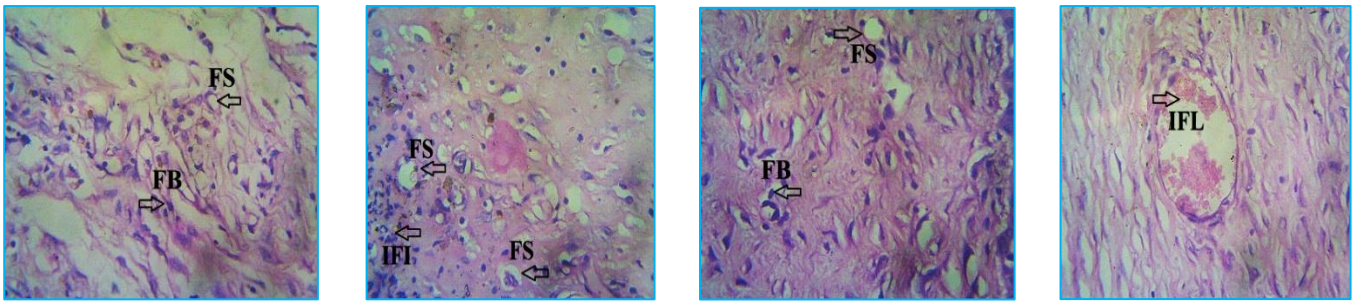
Groups	3rd day	21st day
Control		
Standard PI		
Standard NEO		
LELE oral		
LELE 1%		
LELE 2%		
LEFE oral		
LEFE 1%		
LEFE 2%		
LSLE oral		
LSLE 1%		
LSLE 2%		



I) Control group | II) Standard- PI ointment | III) Standard- Neo-ointment | IV) LELE oral (500mg/kg)



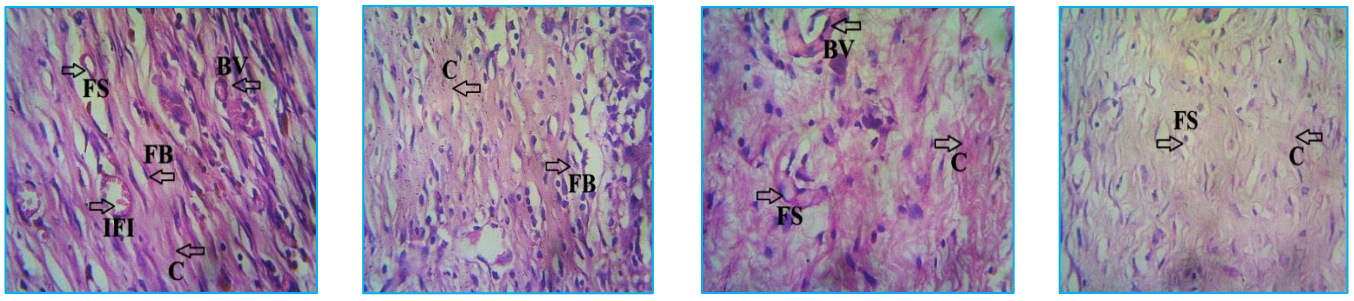
V) LELE 1% ointment | VI) LELE 2% ointment | VII) LEFE oral (500mg/kg) | VIII) LEFE 1% ointment



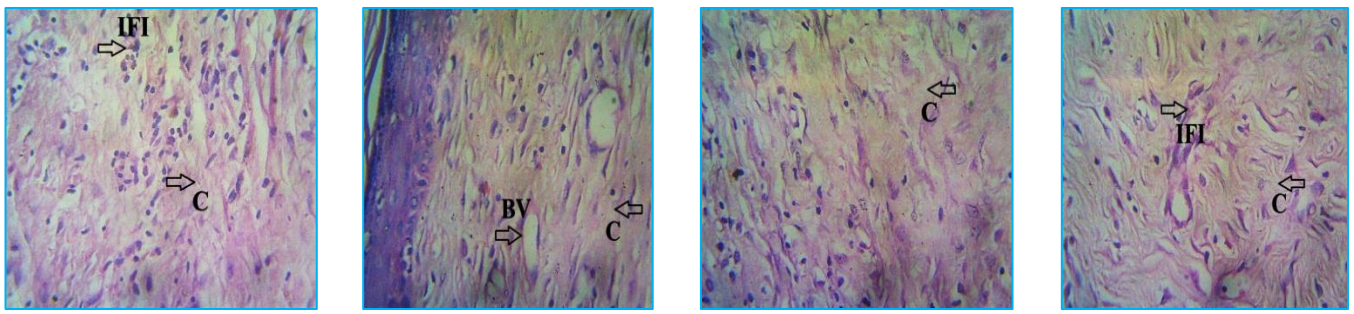
IX) LEFE 2% ointment | X) LESE oral (500mg/kg) | XI) LESE 1% ointment | XII) LESE 2% ointment

PI- Povidone-iodine, **Neo**- Neomycin, **FB**- Fibroblast, **BV**- Blood Vessels, **IFI**- Inflammatory Infiltrates, **FS**- Fibrocollagenous stroma. I) shows fibrocollagenous stroma and inflammatory infiltrates, II) shows re-epithelialization process, III) shows re-epithelialization, IV) shows inflammatory infiltrates, V) shows inflammatory infiltrates in the muscular layer, VI) shows deep dermis with inflammatory infiltrates, VII) shows dermis shows fibrocollagenous stroma, VIII) shows congested vessels, IX) shows deep dermis with inflammatory infiltrates, X) shows fibrocollagenous stroma with inflammatory infiltrates, XI) shows fibrocollagenous stroma, XII) shows fibrocollagenous stroma

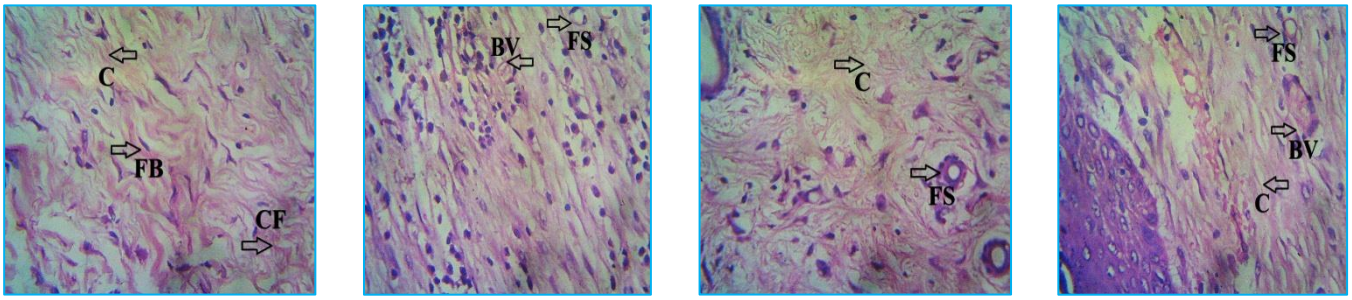
Fig 5 Histology of granulation tissue of wound at 3rd day stained with H&E (40x)



I) Control group | II) Standard- PI ointment | III) Standard- Neo-ointment | IV) LELE oral (500mg/kg)



V) LELE 1% ointment | VI) LELE 2% ointment | VII) LEFE oral (500mg/kg) | VIII) LEFE 1% ointment



IX) LEFE 2% ointment X) LESE oral (500mg/kg) XI) LESE 1% ointment XII) LESE 2% ointment

PI- Povidone-iodine, **Neo**- Neomycin, **FB**- Fibroblast, **BV**- Blood Vessels, **IFI**- Inflammatory Infiltrates, **FS**- Fibrocollagenous stroma. I) dermis shows fibrocollagenous stroma, II) shows dermis with scattered inflammatory infiltrates, III) shows deep dermis with mild inflammatory infiltrates, IV) shows normal epidermis with fibrocollagenous stroma, V) shows deep dermis with inflammatory infiltrates, VI) shows dilated vessels, VII) shows scattered inflammatory infiltrates, VIII) shows fibrocollagenous stroma with thin walled vessels, IX) shows normal epidermis with dermis shows fibrous stroma, X) shows inflammatory infiltrates, XI) shows fibro collagenous stroma with scattered inflammatory infiltrates, XII) shows normal epidermis

Fig 6 Histology of granulation tissue of wound at 21st day stained with H&E (40x)

LITERATURE CITED

1. Tsala DE, Amadou D, Habtemariam S. 2013. Natural wound healing and bioactive natural products. *Phytopharmacology* 4(3): 532-560.
2. Romanelli M, Miteva M, Romanelli P, Barbanera S, Dini V. 2013. Use of diagnostics in wound management. *Curr. Opin. Support. Palliat. Care* 7: 106-110.
3. Planz V, Franzen L, Windbergs M. 2015. Novel in vitro approaches for the simulation and analysis of human skin wounds. *Skin Pharmacology Physiology* 28: 91-96.
4. Hussain F, Islam A, Bulbul L, Moghal MR, Hossain MS. 2014. In vitro thrombolytic potential of root extracts of four medicinal plants available in Bangladesh. *Ancient Science of Life* 33(3): 162-164.
5. Takano J. 2013. *Lagerstroemia speciosa* L. (Banaba or Queen's Flower) - Wonders of Botanical Herbs: Pyroenergen. www.pyroenergen.com/other-products/banabaplant.htm.
6. Nauta AC, Gurtner GC, Longaker MT. 2013. Adult stem cells in small animal wound healing models. In: Gourdie RG, Myers TA, eds, *Wound Regeneration and Repair Methods and Protocols*. New York, NY: Humana Press. pp 81-98.
7. Kongkathip N. 1994. Chemistry and extraction method of neem- Bankon: 3rd workshop in the neem leaf extracts to control and eradicate insects.
8. Harborne JB. 1894. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 2nd Edition, London New York.
9. Trease GE, Evans WC. 1989. *Pharmacognsy*. 11th Edition. Brailliar Tiridel Can, Macmillian Publishers.
10. Koschwanez HE, Broadbent E. 2011. The use of wound healing assessment methods in psychological studies: a review and recommendations. *Br. Jr. Health Psychology* 16: 1-32.
11. Stephens P, Caley M, Peake M. 2013. Alternatives for animal wound model systems. *In*: (Eds) RG Gourdie, TA Myers. *Wound Regeneration and Repair Methods and Protocols*. New York, NY: Humana Press. pp 177- 201.
12. Wong VW, Sorkin M, Glotzbach JP, Longaker MT, Gurtner GC. 2011. Surgical approaches to create murine models of human wound healing. *Jr. Biomed. Biotechnology* 2011: 969618.
13. Gottrup F, Agren MS, Karlsmark T. 2000. Models for use in wound healing research: A survey focusing on in vitro and in vivo adult soft tissue. *Wound Repair Regen*. 8: 83-96.
14. Peplow PV, Chung TY, Baxter GD. 2010. Laser photo biomodulation of proliferation of cells in culture: A review of human and animal studies. *Photomed. Laser Surg*. 28: S3-S40.
15. Abdullah A, Amini-Nik S, Jeschke MG. 2014. Animal models in burn research. *Cell. Mol. Life Science* 71: 3241-3255.
16. Chan EWC, Tan LN, Wong SK. 2014. Phytochemistry and pharmacology of *Lagerstroemia speciosa*: A natural remedy for diabetes. *Int. Jr. Herbal Med.* 2: 100-105.
17. Kolakul P, Fangkrathok N, Kammungkun P, Kranjanasurat P, Sripanidkulchai B. 2013. Antimicrobial effects of Thai plants against acne-inducing bacteria. The 5th Annual Northeast Pharmacy Research Conference on Pharmacy Profession: Moving Forward to ASEAN Harmonization held on 16 to 17th February 2013 at Khon Kaen University, Thailand.
18. Cross DAE, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA. 1995. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature* 378: 785-789.