

Preliminary Investigation of Wound Healing Potential of Leaves and Barks of *Ziziphus oenoplia* using the Cell Migration and Chorioallantoic Membrane Assays

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Abstract

The extracts of leaves and bark of *Ziziphus oenoplia* (L.) Miller (family: Rhamnaceae) (Fig. 01) were subjected to cell migration assay (CMA) and chorioallantoic membrane (CAM) assay to investigate their wound healing potential. The results of those two bioassays provide scientific support for the use of leaves and bark of *Z. oenoplia* for wound healing in traditional medicine in Sri Lanka.

Introduction

The herbal preparations have been used as therapeutic agents for the treatment of wounds in traditional medicine practiced in Sri Lanka. It is reported that the leaves, roots and stem bark of *Z. oenoplia* are used for the treatment of wounds. The proliferation of cells and angiogenesis are key events in the wound healing process¹ that can be enhanced by plant secondary metabolites. The present study is aimed at investigating the wound healing potential of leaves and bark of *Z. oenoplia* using CMA with Madin Darby Canine Kidney (MDCK) cells and CAM assay.

Methodology



Fig.01: *Ziziphus oenoplia*

Extraction

- Dried and powdered stem bark (BK) and leaves (LF) (each 100 g) were extracted sequentially with:
Hexanes (Hex.)
Dichloromethane (DCM)
Ethyl acetate (EtOAc)
Methanol (MeOH)
(each 1.0 L for 24 h × 3)
in an orbital shaker (~90 rpm) at 30±2 °C.
- The solvents were removed under vacuum and weighed.

Cell migration assay (CMA)

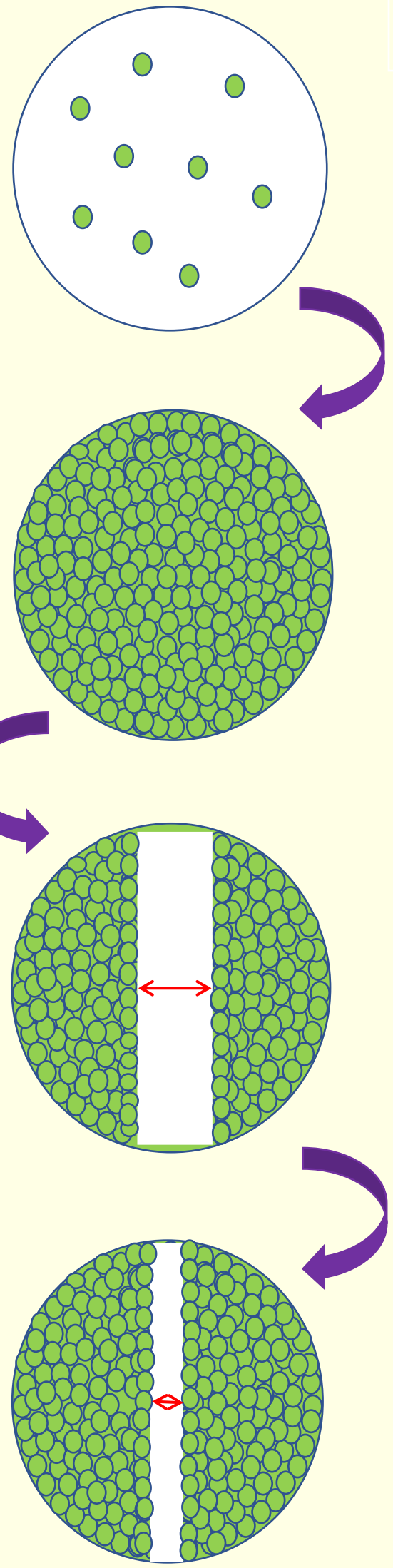
- The cells were inoculated into wells (2.0 × 10⁴ cells/well) in 24 well plates with DMEM and incubated in a humidified incubator for 24 h at 37 °C with 5% CO₂.
- A monolayer of cells was observed under inverted tissue culture microscope (× 400).
- A scratch was made along the vertical axis of each well and washed with phosphate buffered saline (PBS). Each test well was treated with plant extract (20 mg/L). Initial width (at t=0 h) of the scratch was measured and taken as the mean of five readings. The plates treated with test samples and controls were incubated further for 24 h. The CMA was carried out in triplicate.
- After 24 h, the width of the scratch (at t=24 h) was measured and taken as the mean of five readings. The cell migration enhancement was presented as mean percentage wound closure.

Fig.02: Key steps of cell migration assay

Chorioallantoic membrane (CAM) assay

- Fertile chicken eggs were cleaned with 70% ethanol and incubated at 37 °C in a 60% humidified incubator for 9 days with continuous monitoring.
- On the 9th day, a small window (1 cm²) was made in the shell above the vascularized region. Sterile Whatman No:1 paper discs (6 mm) treated with test samples (10 µg in 10 µL of DMSO /disc) were placed on CAM in each egg and the eggs were sealed.
- The eggs treated with test samples and controls were incubated at 37 °C in the 60% humidified incubator for further 3 days.
- On the 12th day, each CAM was harvested, photographed and count of blood vessels in a designated area was presented as the mean vascular index² with five replicates.

Fig.03: Key steps of chorioallantoic membrane assay



Statistical Analysis

Mean comparisons were performed using ANOVA and the least significant test (LSD) with significance level of $p \leq 0.05$ using the SAS Ver. 9 (1993).

Results and Discussion

- A significant enhancement of cell migration was shown by hexanes extracts of bark, and hexanes, dichloromethane and ethyl acetate extracts of leaves of *Z. oenoplia* (Fig. 04 and 05).

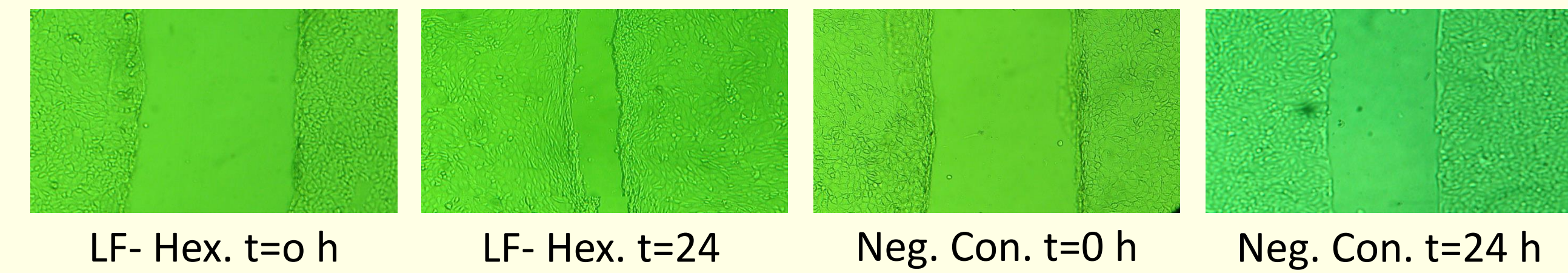


Fig 04: Microscopic images (× 400) of the wounds in the monolayer of cells at the beginning (t=0 h) and after 24 h (t=24 h) treating with hexanes extract of leaves (LF-Hex.) (20 mg/L) with a negative control (Neg. Con.-1%DMSO in 20% DMEM)

- A higher mean vascular index was shown by hexanes and methanol extracts of bark, and ethyl acetate and methanol extracts of leaves of *Z. oenoplia* (Fig.06 and 07).

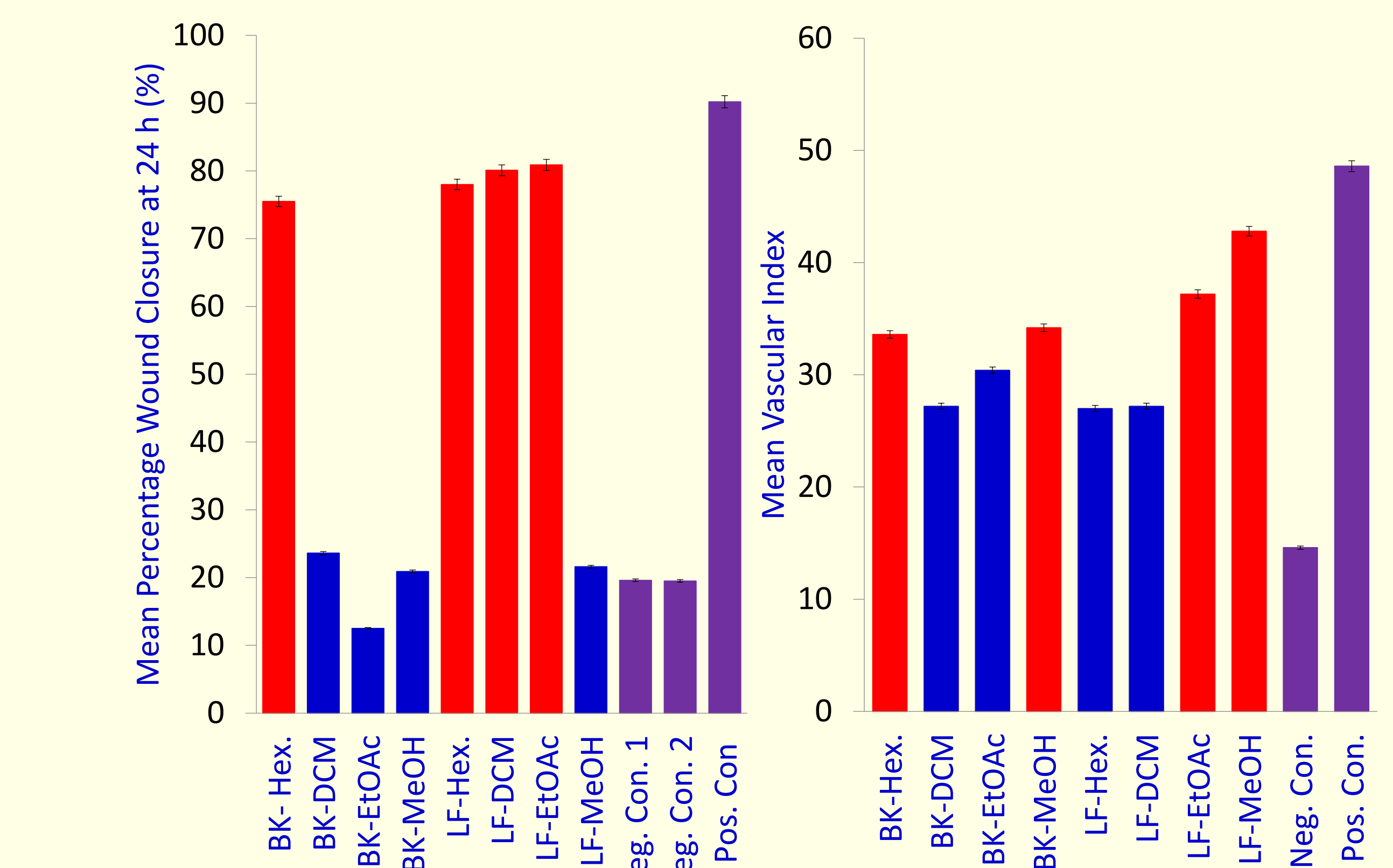


Fig.05: Mean percentage wound closure for each extract with controls.

Neg. Con. 1-20% DMEM, Neg. Con. 2-1% DMSO in 20% DMEM, Pos. Con.- Asiaticoside (25 µM)

Fig.06: Mean vascular index for each extract with controls.

Neg. Con. - DMSO (10 µL/Disc), Pos. Con.- β-Sitosterol (10 µg/Disc).

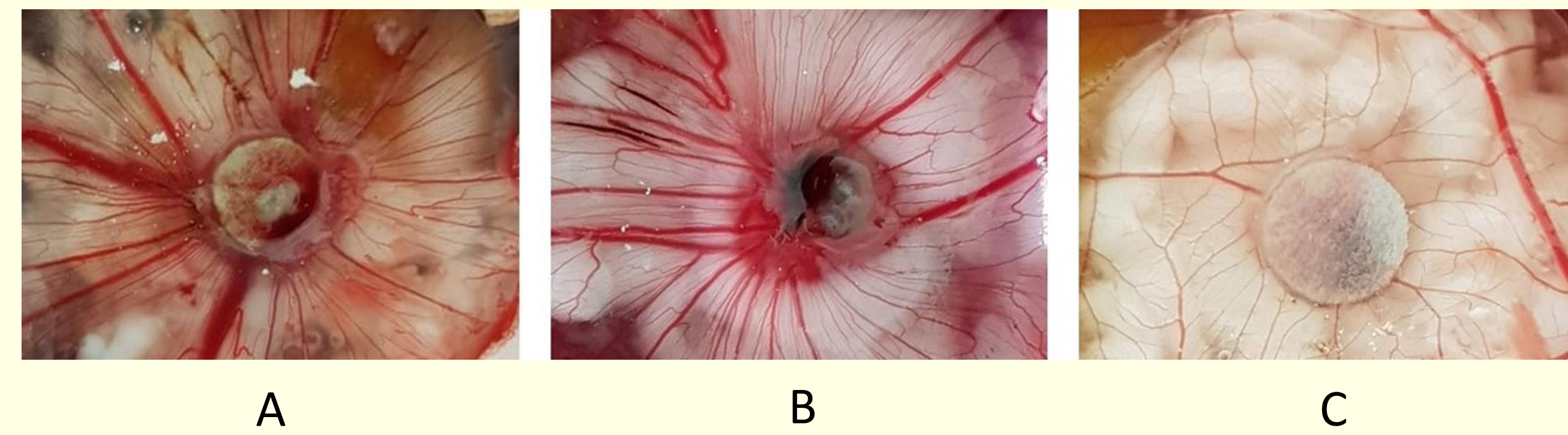


Fig. 07: Images of CAM Vascularization after 72 h of Incubation treated with (A) LF-MeOH, (B) LF-EtOAc extracts (10 µg/disc) and (C) DMSO (negative control) (10 µL/disc)

Conclusion

- Hexanes extracts of bark, and hexanes, dichloromethane and ethyl acetate extracts of leaves of *Z. oenoplia* exhibit cell migration activity.
- Hexanes and methanol extracts of bark, and ethyl acetate and methanol extracts of leaves of *Z. oenoplia* exhibit angiogenic activity.
- The present study partially provides scientific support for the use of *Z. oenoplia* leaves and bark in traditional medicine in Sri Lanka for wound healing.
- The chemical investigation of the active extracts is underway to identify the active compounds.

References

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Abbreviations: DMEM - Dulbecco's Modified Eagle Medium
DMSO - Dimethyl sulfoxide