

How to Cite:

Soja, A., & Jayakumari, S. (2022). In vitro and in vivo studies on the wound healing activity of leaf extracts of *Homalium zeylanicum* benth. *International Journal of Health Sciences*, 6(S4), 4055–4063. <https://doi.org/10.53730/ijhs.v6nS4.9033>

In vitro and in vivo studies on the wound healing activity of leaf extracts of *Homalium zeylanicum* benth

Soja A

Assistant Professor, College of Pharmaceutical Sciences, Government Medical College, Thiruvananthapuram

Corresponding author email: sojakrishnan2007@yahoo.co.in

Dr S Jayakumari

Professor and Head, Department of Pharmacognosy, School of Pharmaceutical Sciences, Vels Institute of Science Technology and Advanced Studies, Pallavaram, Chennai

Abstract---In this study, the Indian medicinal herb *Homalium zeylanicum* benth was tested for its ability to cure wounds. A variety of solvents have been used to extract the leaves of the plant. Leaf extract was used for wound healing research both in the laboratory and in animals. HaCaT cells were used to investigate the cytotoxicity of plant extracts, and the IC₅₀ values for the various leaf ethyl acetate extracts were determined. All doses of the test samples indicated reduced cytotoxicity on the tested cell line. Scratch assays were used to measure the migration rates of HaCaT cells. During an in vivo study, animals treated with ethyl acetate leaf extract had higher percentages of wound contraction power and tensile strength.. According to this study, the plant's leaf extract may have wound-healing properties, which is in line with its traditional medicinal uses.

Keywords---homalium zeylanicum, leaf extract, wound healing activity, cell line.

Introduction

Restoring the wounded tissue to its pre-injury state is the goal of wound healing. A wound's healing process includes several stages, including inflammation, proliferation, and wound repair. Blood clotting is the first step in the healing process, which is then completed by redesigning the skin's cellular layers. ROS (Reactive oxygen species) and bacteria can impede wound healing, obscuring the process. Cells that have been injured or lost

their identity will be first identified at the site of the injury. Antimicrobial defence is blamed for the cell damage, which is caused by the peroxidation of membrane lipids [2]. In order to restore the skin's anatomical and functional integrity, suitable wound healing treatments must be used. Herbs have been used for centuries by traditional healers around the world, particularly in India and China.[4].

Numerous medicinal plants and their formulations have been used topically to heal wounds since ancient times. The search for an alternative to contemporary wound healing treatments has spread to more prosperous countries as well, as well. Healing wounds with herbs is becoming increasingly popular. A wide range of Indian medicinal plants are in use across the globe. There are Flacourtiaceae in the Salicaceae family, and there is a member called *Homalium ceylanicum* Benth (Syn. *H. zeylanicum*)[5] (Salicaceae). One of the largest evergreen trees in the world, *Homalium ceylanicum* can be found only in South India and Sri Lanka.

In this case, the number seven. *Blackwellia ceylanica*, also known as liyan, mukki, and. Analgesics, anti-inflammatory agents, anthelmintics, antioxidants, cytotoxins, wound healing and anti-rheumatic properties are just a few of the numerous traditional therapeutic applications for this plant's bark and leaves. In addition to its anti-inflammatory, anti-inflammatory, and hepatoprotective properties, it has also been linked to anti-diabetic effects. Aphrodisiac and a traditional medicine in Nigeria, it treats malaria, ulcers, and other inflammatory and autoimmune illnesses. Various extracts of *Homalium zeylanicum* have been characterised as anthelmintic, anti-diabetic, anti-dyslipidemic, and hepatoprotective conditioning [8-12]. Thenmala Hills in Kerala's Kollam district provided the plant specimens for the proposed research. It was the goal of the current research to determine whether or not ethyl acetate leaf extract could repair wounds in rats, both in cell line models and in actual circular and incision wound models.

Materials and Methods

Plant Collection

When gathering plant samples for this study, a local taxonomist from Kerala, India, offered a hand. The Pharmacognosist of the Institute has certified and registered the PARC/2018/3750 registration.

Extraction

A 40-mesh filter was used to separate the plant's fresh healthy leaves after they had been verified and dried in the shade for three weeks. Soxhlet extractions of air-dried leaf portions of the plant yielded around 1 kg of powdered plant material kept in a closed bag. The dried weight of the plant material was used to weigh the extracts made with each of the solvents, and the yield % was calculated.

Cytotoxicity assay

Preparation of cell suspension

DMEM-grown HaCaT cells were trypsinized in a separate experiment, after discarding the growth media. In a flask with 25 mL of DMEM containing 10% FCS, cells that had been disintegrated were added. A gentle pipette passage was used to homogenise the cells after they had been suspended in the liquid. Cell grafts from the veins Before collecting the plant extract, the 96-well culture plate was diluted to concentrations ranging from 0 to 400 grammes of plant extract per millilitre before incubating at 37°C for 24 hours. A culture microscope was used to examine the cells after a 48-hour incubation period. The cytotoxic testing was conducted out with 80% confluence of cells.

Cytotoxicity assay

At least one of the following was tested in this experiment: 3, 4(4, 5 dimethyl thiazol-2yl) 2, 5 diphenyltetrazolium bromide (MTT). A purple formazan product is formed when cells containing mitochondrial Succinate dehydrogenase and reductase bind to MTT. The cytotoxicity of this formazan product is directly related to the number of cells that can be killed. Wells were treated with MTT and incubated for an additional three hours after 48 hours of incubation. To dissolve the formazan particles, 100 µl of DMSO/SDS solution was used, and a Lark LIPR-9608 microplate reader was used to detect the absorbance at 540 nm.^[13]

In Vitro Wound Scratch Assay

The scratch assay technique was used to measure the migration rates of HaCaT cells [14]. 2×10^5 cells were sown into each well of a 24-well plate and cultured for 24 hours in a humid atmosphere at 37°C with 5 percent CO₂. It was necessary to scrape the monolayer of confluent cells horizontally after it had been incubated for 24 hours with a sterile 200-ml pipette tip. PBS was used to wash away the remaining debris. Lacing the cells with serum-free DMEM allowed the standard and test compounds to reach levels of 100 g/mL in the cells. The control cell was one that had not been altered in any way. Microscopy was used to capture images of a scratch that represented the crack at 0 hours before the test and reference chemicals were kept at 40 exaggeration. This happened after 24 and 48 hours of incubation, respectively. Anatomized photos were used to calculate the migration rate by matching the closed area's probability to its value at 0 hours. Cell migration was suggested by an increase in the closed region's frequency.

In Vivo Study

Animals

During this research, rats weighing between 200 and 250 grams were employed. The rats were always fed commercial rat food and water from M.S. Hindustan lever limited in Bangalore, India. CBLRC/IAEC/03/01-2021 was the clearance number given to the research by the Institutional Animal Ethical Committee,

which approved the research. Excision wound models required the use of six animals per group, with three animals per group undergoing the procedure. Since Group G1 was used as a control group, additional groups (G2, G3, and 4) were used as either test or control groups, with the right therapy applied to each of these groupings.

Preparation of formulation and standard used

Ointments with 5 mg/ μ g and 10 mg/ μ g of ethyl acetate leaf extract were made with S.D. Fine chemicals, India, white soft paraffin. Base and leaf extract were mixed to produce this 500mg dose. Ointment from India's Rixin pharmaceutical company, 10mg/g, was utilised as the standard treatment for wound healing in various animal models.

Evaluation of wound healing activity by excision wound model

A moderate ether anaesthetic was used to cut circular incisions of roughly 10mm diameter into the skin that had been cleansed. Placing a sheet of translucent paper on top of a wound instantly traced the wound's area (in square millimetres). The tracing was transferred to graph paper and used to determine the size of the wound. This will be the initial readout on the wound area. G1 was given by applying a simple ointment I.P. to the area affected. Topical application of a 5 mg/ μ g ointment containing Ethyl acetate leaf extract was used to give G2. Ointment containing 10 mg/g leaf extract of ethyl acetate in Group 3 Applying the 10mg/g silver sulfadiazine ointment to the area affected. After 15 days of twice-daily application of all the samples, researchers examined at how much the wound had grown. On days 2, 4, 6, 8, 10, 12, and 14, after each animal was injured, the wound area was measured.. The acute healing process came to an end on Day 14. A wound contraction percentage was determined using this formula:

$$\% \text{ of wound contraction} = \frac{\text{Initial wound size} - \text{Specific day wound size}}{\text{Initial wound size}} \times 100$$

Estimation of wound healing activity by incision wound model

[16] The procedure for creating an incision wound was followed. Similar to the excision wound model, animals were separated into groups and given the same treatments. To put the rats to sleep, we administered 50 mg/kg of i.p. ketamine hydrochloride per kilogram body weight. After shaving the rats, a sharp scalpel was used to make a 6 cm-long paravertebral incision on both sides of the vertebral column using the complete skin thickness. A twisted surgical needle and black silk surgical thread (number 000) were used to establish hemostasis before interrupted sutures were used to close the incision (number 11).

Statistical analysis

The wound model data were provided as mean \pm SEM for the treatment and control groups. An ANOVA and a Dunnett's Multiple Comparative Test were used

to compare the treatment and control groups, respectively. The significance of a P value of 0.05 or less was established.

Results

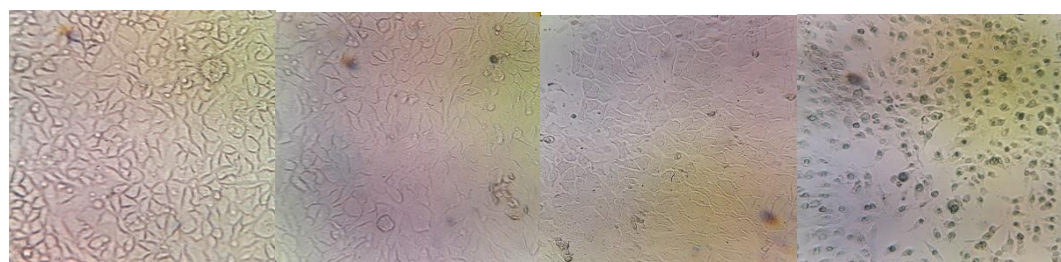
Cytotoxicity Assay

With increasing sample concentrations, a small decrease in HaCaT cell cytotoxicity was seen, although this was verified in trials using the plant extract (Table 1). The materials tested showed cytotoxic activity against HaCaT cells of up to 56.50 percent at a dose of 400 $\mu\text{g}/\text{mL}$. High cytotoxicity samples did not cause cell disintegration for 48 hours at higher doses (Figure 1). According to the plant extract's IC₅₀, it is 403.43 $\mu\text{g}/\text{mL}$ (Figure 2).

Table 1. In vitro cytotoxicity effect of leaf extracts against HaCaT cells lines

Sample Concentration	% Cell Viability
0	100.00
6.25	98.91
12	92.62
25	80.63
50	71.82
100	70.50
200	65.14
400	56.50

Figure 1: Cytotoxicity effect of Leaf extract against HaCaT Cell lines

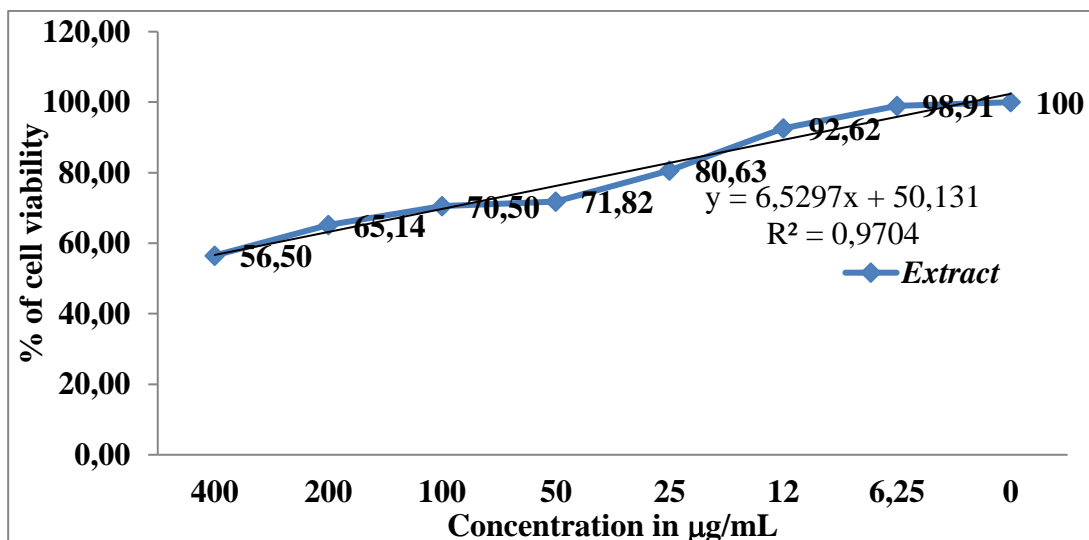


Control
Conc: 400 $\mu\text{g}/\text{mL}$

Conc: 100 $\mu\text{g}/\text{mL}$

Conc: 200 $\mu\text{g}/\text{mL}$

Figure 2: Cytotoxicity effect of Leaf extract against HaCaT Cell lines



Wound Healing Assay

Studies on wound healing in vitro show that cell migration is critical for fracture repair. For cell scratch assays, cell viability investigations were performed to identify the lowest active concentration, which was used to estimate the wound healing capacity of test samples. The cell migrates to the generated provisional gap after being exposed to the test materials for 24 and 48 hours. For the 24 and 48-hour measurements, the scratch coverage rate was used to arrive at the final results. Scratch control (0 h) images were exhibited (Figure 3) in comparison to plant extract. The plant extract sample revealed a cell migration rate of 45.28 percent after 24 hours and a cell migration rate of 96.99 percent after 48 hours (Figure 4)

Figure 3: In vitro scratch assay migration on cells were scratched and treated with and without Plant extract treatment

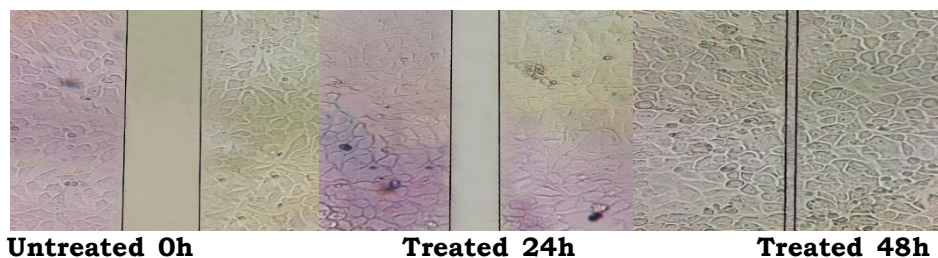
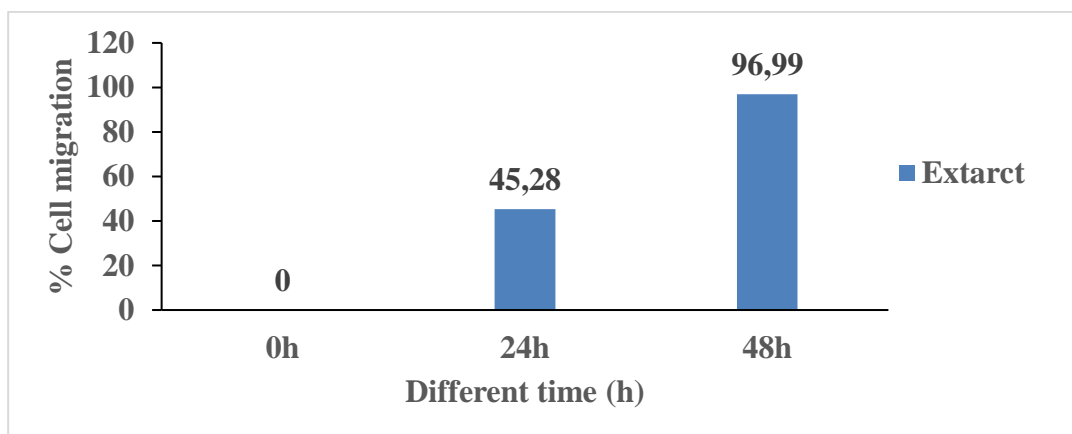


Figure 4: In vitro scratch assay migration percentage on cells were scratched and treated with plant extract



In vivo study

Test samples were evaluated using the Excision wound model, which simulates wound closure and contraction, as well as the time it takes for epithelialization. During the 14-day comment period, the impact was monitored every two days. To compare, when tested on the 14th day after a wound incision, standard medicine silver sulfadiazine 10mg/g showed a healing rate of 99.82%, while the test extracts of ethyl acetate leaf extract 5 mg/g and 10mg/g showed healing rates of 94.24% and 97.55%. Table 2 (a) When compared to a control group, mice treated with Ethyl acetate leaf extract showed a substantial (* $p < 0.05$) increase in the percentage of wound healing power. In the Incision wound model, there were no bandages on the wounds, and the animals got treatment every day for 10 days. Following anaesthesia, the tensile strength of the treated wound skin was measured using a tensiometer on the tenth day. Using the Homalium zeylanicum leaf extract, the contraction and strength of hematoma wounds were enhanced ($P < 0.05$). (Table 3)

Table 2. Effect of Ethyl acetate leaf extract on wound contraction

Post wounding days	Dose mg/g	Percentage of wound contraction						
		2	4	6	8	10	12	14
Control	Simple ointment	5.32±1.79	22.7±11.77	31.12±4.06	45.7±9.43	66.47±6.60	82.97±6.17	86.02±3.10
Ethyl acetate leaf extract	5	7.95±1.66	18.45±1.84	29.05±5.27	42.37±7.15	72.75±19.48	84.22±5.58*	94.27±3.87*
Ethyl acetate leaf extract	10	9.65±3.26	16.32±10.66	33.95±11.61	49.52±12.97	67.52±9.53*	84.17±4.17*	97.75±3.65**
Silver	10	13.3±8.	22.95±7.1	28.75±7.7	47.27±6.1	67.27±5.94	77.87±5.70	99.82±0

sulfadiazine		45	0	4	4*	**	**	.80**
--------------	--	----	---	---	----	----	----	-------

Wound contraction increased with doses of 5 and 10 mg/g. '

Values reported as mean \pm SEM (n=6). A one-way ANOVA and a multiple comparison test with Dunnett's adjustment were used to examine the data. When compared to the control group, * p <0.05 and ** p <0.01 are statistically significant, with ** p <0.01 indicating even greater significance than * p <0.05.

Groups	Wound breaking strength in grams
Group I Control	156.48 \pm 3.96
Group II Ethyl acetate leaf extract 5 mg/g ointment applied topically.	191.65 \pm 4.10**
Group III Ethyl acetate leaf extract 10 mg/g ointment applied topically.	199.74 \pm 4.58**
Group IV Applying the 10mg/g silver sulfadiazine ointment to the affected area.	208.32 \pm 5.38**

Table 3 Effect of ethyl acetate leaf extract on tensile strength of wound in incision wound model

Discussion and Conclusion

Inflammation, proliferation, and remodelling all have a role in wound healing, which is a normal reaction of wounded skin. Irritation, which occurs as a defensive mechanism for tissue, is the initial response during the healing stage. [17] Excision wound models were utilised to examine the healing activities of the wounds. For the treatment of wounds, India has 64 ethnomedical herbs from which to choose [4]. Experiments were conducted to investigate if the ethyl acetate portion of *Homalium Zeylanicum* leaf extracts might speed up wound healing in HaCaT cells *in vitro* and *in vivo* wound healing models, results suggested that the extract was beneficial. Researchers found flavonoids tannins and alkaloids in extracts, which are known promoters of wound healing. Hence HZ Ethyl Acetate Leaf Extract could be suggested as a possible Wound Healing Agent.

References

1. Gupta, A., Singh, R.L., and Raghubir, R. Antioxidant status during cutaneous wound healing in immunocompromised rats. *Mol. Cell. Biochem.*, 2002, 241:1-7.
2. Russo, A., Longo, R., and Vanella, A. Antioxidant activity of propolis: role of caffeic acid phenethyl ester and galangin. *Fitoterapia.*, 2002, 73: S21-S29.
3. Meenakshi, S., Raghavan, G., Virendra, N., Ajay Kumar, S.R., and Shanta, M. Antimicrobial, wound healing and antioxidant activity of *Plagiochasma appendiculatum*. *Journal of Ethnopharmacology.*, 2006, 107:67-72.
4. Kumar, B., Vijay kumar, M., Govindarajan, R., and Pushpangadan, P. Ethanopharmacological approaches to wound healing- exploring medicinal plants of india. *Journal of Ethnopharmacology.*, 2007, 114:103-113.
5. International plant name index [homepage on internet]. 2004. Available from:

- <http://www.ipni.org/ipni/idplantnamesearch.do>.
6. Sue Zmarzty Don Krikup, herbarium, Kew. Interactive Key to Genera of Salicaceae sensu lato and Achariaceae sensu lato. www.kew.org/herbarium/keys/flacs/_baks/index.html.0006.d323.bak.
 7. Sililan K. Homaliumzeylanicum (Gardner) Benth. J Linn Soc Bot. 1859;(4):35
 8. SwathiPothireddy, International Journal of Research and Development in Pharmacy and Life Sciences, 2014;3(3):1004-1010.
 9. Rachalavinodkumar, phytochemical constituents and biological activities of Homaliumzeylanicum Benth, Int J Pharm Bio Sci, 2014;5(3):176 – 182
 10. K. Siva Sankara Prasad, assessment of the analgesic and anti-inflammatory effect of Homaliumzeylanicum, JGTPS, 2014;5(3): 1886 – 1890
 11. Kumar dileep, Clinical evolution of Sarpagandhachurna in the management of Yoshaapasmara vis-à-vis hysterical neurosis, IRJP, 2014;5(3):174-177.
 12. Natava Rajesh, In -vitro and In-vivo Studies on the Antidiabetic Activity of Stem Bark of Homaliumzeylanicum in STZ Induced Diabetic Rats, Asian Journal of Biochemical and Pharmaceutical Research, 2014;4(3):76-90.
 13. Tim Mosmann. Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. Journal of Immunological Methods, 65 (1983) 55-63.
 14. M. Harishkumar, Y. Masatoshi, S. Hiroshi, et al., Revealing the mechanism of in vitro wound healing properties of citrus tamurana extract, BioMed Res. Int. (2013) 2013
 15. Cooper., and Gunn's. In: Carter, S.L. (Ed.), Dispensing for Pharmaceutical Students, 12th ed. CBS Publisher and Distributors, Delhi, 1987, 199–200.
 16. S. Hemalata, N. Subramanian, V. Ravichandran, and K. Chinnaswamy, "Wound healing activity of Indigofera encephalylla Linn," Indian Journal of Pharmaceutical Sciences, vol. 63, no. 4, pp. 331–333, 2001.
 17. Kondo, T., Timing of skin wounds. *Legal Medicine.*, 2007, 9, 109–114.
 18. Arnawa, I.K., Sapanca, P.L.Y., Martini, L.K.B., Udayana, I.G.B., Suryasa, W. (2019). Food security program towards community food consumption. *Journal of Advanced Research in Dynamical and Control Systems*, 11(2), 1198-1210.
 19. Damayanti, I. A. M., Indrayoni, P., Antari, N. W. S., & Padmiswari, A. A. I. M. (2021). Effectiveness of Averrhoa bilimbi leaf extract on spermatogenic cells of mice (Mus Musculus L.) hyperglycemia. *International Journal of Health & Medical Sciences*, 4(2), 273-279. <https://doi.org/10.21744/ijhms.v4n2.1747>