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Phytochemical Screening, TLC Profiling, and in Vitro Wound Healing Evaluation of Hydroalcoholic Leaf Extract of Jatropha Integerrima

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Abstract: *The present study aimed to evaluate the in vitro wound healing activity of hydroalcoholic leaf extract of Jatropha integerrima through assessment of cell migration, antioxidant, and antimicrobial properties. Fresh leaves were collected, authenticated, shade-dried, powdered, and extracted by the maceration method using a hydroalcoholic solvent. Preliminary phytochemical screening revealed the presence of flavonoids, tannins, phenols, saponins, terpenoids, and alkaloids. Thin Layer Chromatography (TLC) analysis showed distinct phytochemical constituents with Rf values of 0.58 and 0.25. Wound healing activity was evaluated using an in vitro scratch assay on L929 fibroblast cells. The extract demonstrated significant cell migration and wound closure, achieving 74.07% wound healing activity after 48 hours compared with 87.53% for the standard drug Cipladine. The findings suggest that Jatropha integerrima leaf extract possesses promising wound healing potential and may serve as a natural therapeutic agent for tissue repair and regeneration.*

Keywords: Jatropha integerrima, Wound Healing, Scratch Assay, Phytochemical Screening, Cell Migration

I. INTRODUCTION

Wound healing is a complex and dynamic biological process that restores the integrity and function of damaged tissues following injury. It involves a series of overlapping phases, including hemostasis, inflammation, proliferation, and remodeling. During the inflammatory phase, immune cells remove debris and pathogens from the wound site. The proliferative phase is characterized by fibroblast migration, collagen synthesis, angiogenesis, and re-epithelialization, while the remodeling phase strengthens the newly formed tissue through extracellular matrix reorganization. Impairment of any of these stages may delay wound healing and increase the risk of infection and chronic wound formation.[1-2] The prevalence of chronic wounds, burns, diabetic ulcers, and traumatic injuries has increased significantly worldwide, creating a substantial healthcare burden. Conventional wound management strategies involve the use of antiseptics, antibiotics, dressings, and growth factor-based therapies. However, limitations such as high treatment costs, microbial resistance, adverse effects, and inadequate healing outcomes have prompted the search for safer and more effective alternatives.[3-4] Medicinal plants have attracted considerable attention due to their rich content of bioactive compounds and long history of traditional use in wound management.

Plant-derived phytochemicals such as flavonoids, phenolic compounds, tannins, alkaloids, terpenoids, and saponins possess antioxidant, anti-inflammatory, antimicrobial, and tissue regenerative properties that contribute to wound healing. These compounds help reduce oxidative stress, prevent microbial infection, stimulate fibroblast proliferation, and enhance collagen deposition, thereby accelerating the wound repair process.[5-6] Consequently, medicinal plants are increasingly being investigated as potential sources of novel wound healing agents. *Jatropha integerrima* Jacq., commonly known as peregrina or spicy jatropha, belongs to the family Euphorbiaceae. The plant is widely distributed

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in tropical and subtropical regions and is cultivated as an ornamental shrub due to its attractive flowers. Various species of the genus *Jatropha* have been traditionally used for the treatment of wounds, skin infections, inflammation, fever, and other ailments. Previous phytochemical investigations have revealed that *Jatropha* species contain diverse bioactive constituents, including flavonoids, diterpenoids, alkaloids, tannins, and phenolic compounds, which exhibit significant biological activities.[7-8]

Several studies have reported antimicrobial, antioxidant, anti-inflammatory, and cytoprotective properties of *Jatropha* species. These pharmacological activities are closely associated with wound healing because effective wound repair requires the control of microbial growth, reduction of oxidative damage, and stimulation of cellular regeneration. Antioxidants neutralize reactive oxygen species generated at the wound site, thereby preventing cellular damage and promoting tissue recovery. Similarly, antimicrobial agents help prevent infection, which is one of the major causes of delayed wound healing.[9-10]

In vitro wound healing models have become valuable tools for the preliminary evaluation of wound healing activity. Among these, the scratch assay is widely used to assess cell migration and proliferation, two critical events involved in tissue regeneration. In this method, an artificial scratch is created on a confluent monolayer of cells, and the rate of wound closure is monitored over time. Increased cell migration toward the wound area indicates enhanced healing potential of the test sample.[11-12]

Phytochemical screening and chromatographic techniques such as Thin Layer Chromatography (TLC) play an important role in identifying and characterizing the bioactive constituents responsible for therapeutic activity. TLC serves as a simple and cost-effective method for obtaining a phytochemical fingerprint of plant extracts and helps in the preliminary identification of compounds that may contribute to wound healing activity.[13]

The present study was undertaken to investigate the in vitro wound healing activity of the hydroalcoholic leaf extract of *Jatropha integerrima*. The study included phytochemical screening, TLC analysis, and evaluation of cell migration using an in vitro scratch assay on L929 fibroblast cells. The findings may provide scientific evidence supporting the therapeutic potential of *Jatropha integerrima* as a natural source for wound healing and tissue regeneration applications.

II. MATERIAL AND METHOD

Fresh leaves of Jatropha integerrima were collected from the campus of Nootan College of Pharmacy, Kavathe Mahankal, Maharashtra, India. The leaves were washed with distilled water, shade-dried, and coarsely powdered. The powdered material was stored in an airtight container for further studies. Plant authentication was carried out by the Department of Botany, PVP College of Arts, Science, and Commerce, Kavathe Mahankal, and the authenticated material was used for subsequent phytochemical and wound healing investigations.

Extraction (Maceration)

The powdered leaves of Jatropha integerrima were extracted by the maceration method using a hydroalcoholic solvent (ethanol:water, 1:10). The powder was soaked at room temperature with occasional stirring, followed by filtration through muslin cloth and Whatman filter paper. The filtrate was concentrated by solvent evaporation to obtain a semisolid extract, which was stored in an airtight container under refrigerated conditions for further studies.[14]

Phytochemical Screening:[15]

1. **Detection of Flavonoids:**
   a. **Shinoda Test:** 2 ml extract + Mg ribbon + few drops conc. HCl → Pink/red colour indicates flavonoids
2. **Detection of Tannins:**
   a. **Ferric Chloride Test:** 2ml extract + few drops $FeCl_3$ → Blue-black/green colour indicates tannins
3. **Detection of Saponins:**
   a. **Foam Test:** 2 ml extract + 5 ml water (shake) → Persistent foam indicates saponins

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4. **Detection of Phenols**:
   a. **Ferric Chloride Test:** 2 ml extract + few drops $FeCl_3$ → Dark blue/black colour indicates phenols
5. **Detection of Terpenoids:**
   a. **Salkowski Test:** 2 ml chloroform + 2 ml extract + 3 ml conc. H_2SO_4 → Reddish-brown layer indicates terpenoids
6. **Detection of Alkaloids:a.**
   a. **Mayer's Test:** 2 ml extract + Mayer's reagent → Cream precipitate indicates alkaloids

In Vitro Scratch Assay Method:[16]

The wound healing capabilities of Sample was assayed by performing In vitro cell migration studies on L929 cells by a previously described method. Briefly, 2 x 105 cells/mL were seeded in 6- well plates and were cultured overnight. Cells were then washed with Dulbucco's Phosphate Buffered Saline (DPBS) and a scratch was made with a sterile 200µL tip. The detached cells and other cellular debris were removed by washing the cells with DPBS. The cells were treated with 100 µL of Sample and 5 µg/mL of positive control, Cipladine and incubated for 24 h. Cipladine is a standard drug that is used in wound healing. Untreated cells were negative control. The cell migration and morphological changes of cells were observed in the images taken by inverted microscope, equipped with digital camera. The experiments were performed in triplicate (n ¼ 3). The width of the scratch and wound closure at different time intervals (48hrs) was analyzed by SAGLO software.

Calculations:

Percentage (%) of Wound Closure/ Cell Migration

$$\text{Percentage of Wound Closure} = \frac{\text{Initial Wound Width-Final Wound Width}}{\text{Initial Wound Width}} \times 100$$

Result

Authentication of Plant Material

The collected plant material of Jatropha integerrima was authenticated by the Department of Botany, PVP College of Arts, Science, and Commerce, Kavathe Mahankal, Maharashtra, India. The authenticated specimen was used for all subsequent experimental studies.

Phytochemical analysis

The hydroalcoholic leaf extract of Jatropha integerrima was subjected to preliminary phytochemical screening using standard qualitative tests to identify the presence of various phytoconstituents. The extract was found to contain alkaloids, flavonoids, phenolic compounds, tannins, saponins, glycosides, carbohydrates, proteins, and terpenoids, which may contribute to its biological and wound healing activities.

Table No. 1 Phytochemical analysis by extract

Sr. No	Phytochemicals	Test Used	Result
1.	Flavonoids	Shinoda Test	Present
2.	Tannins	Ferric Chloride Test	Present
3.	Saponins	Foam Test	Present
4.	Phenols	Ferric Chloride Test	Present
5.	Terpenoids	Salkowski Test	Present
6.	Alkaloids	Mayer's Test	Present

Maceration Extraction Procedure

Maceration is a simple and widely used method for extracting active constituents from plant materials. In this method, the powdered plant material is soaked in a suitable solvent for a specific period to dissolve the bioactive compounds. It is commonly used in herbal research because it is easy, cost-effective, and does not require special equipment.

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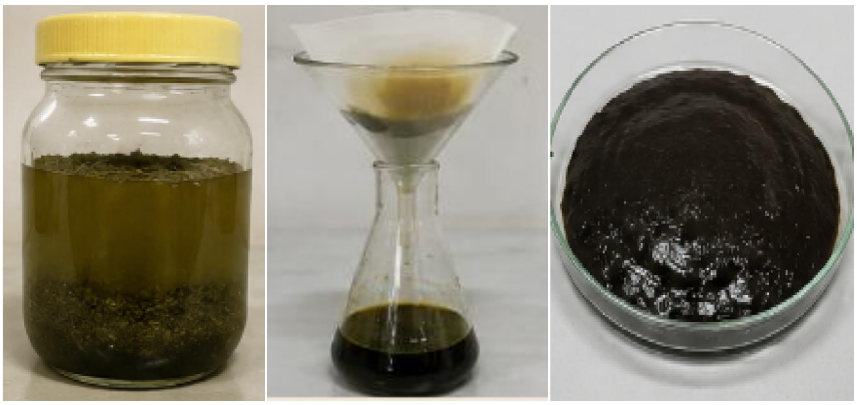

Fig. No. 1. Extraction Process of *Jatropha integerrima* Leaves: Maceration, Filtration, and Semisolid Extract Formation TLC

Thin Layer Chromatography (TLC) was performed to separate and identify the phytochemical constituents present in the hydroalcoholic extract of *Jatropha integerrima* leaves. Silica gel 60 F254 precoated TLC plates were used as the stationary phase. The extract was dissolved in a suitable solvent and spotted on the TLC plate using a capillary tube. The plate was developed in a TLC chamber saturated with an appropriate mobile phase.

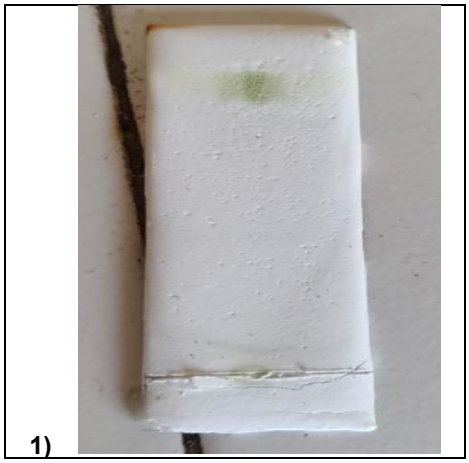

Fig. 2. TLC analysis of *Jatropha integerrima* leaf extract

2) Spot	3) Distance (cm)	4) Rf Value
5) Upper spot	6) 4.6	7) 0.58
8) Lower spot	9) 2.0	10) 0.25

Rf = Distance traveled by compound / Distance traveled by solvent front

Assessment of Cell Migration and Wound Healing (%)

Table 2 .Percentage (%) of cells migrated towards the wound and involved in wound closure

Sample	Reading 0 hours (mm)	Mean	Reading 48 hours (mm)	Mean	% of Cell Migration
Control	10.00		9.79		-
	09.98	10.01	9.80	9.76	
	10.05		9.70		

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tandard-	10.00		1.22		
Cipladine	09.98	10.01	1.20	1.22	87.53
(µg/mL)	10.05		1.24		
ple –Extract	10.00		2.40		
	09.98	10.01	2.60	2.53	74.07
	10.05		2.60		

Calculation:

By Observed Data:

Initial Wound Width (0 hr) = 10.01 mm., Final Wound Width (48 hr) = 2.53 mm

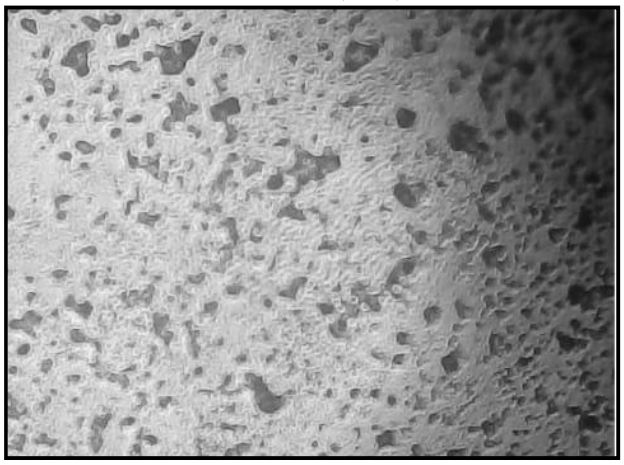

Fig. 3 Normal Morphology of L929

The hydroalcoholic extract of Jatropha integerrima demonstrated significant wound healing activity in the in vitro scratch assay. The extract showed 74.07% cell migration and wound closure after 48 hours, indicating its ability to promote fibroblast migration and tissue repair. Although the activity was slightly lower than the standard drug Cipladine (87.53%), the results suggest that the extract possesses promising wound healing potential and may serve as a natural source for the development of herbal wound healing formulations.

III. CONCLUSION

The present investigation demonstrated the significant wound healing potential of the hydroalcoholic leaf extract of *Jatropha integerrima*. Preliminary phytochemical screening confirmed the presence of several bioactive constituents, including flavonoids, tannins, phenols, saponins, terpenoids, and alkaloids, which are known to contribute to antioxidant, antimicrobial, and tissue regenerative activities. TLC analysis further supported the presence of multiple phytochemical compounds within the extract. The in vitro scratch assay performed on L929 fibroblast cells revealed considerable enhancement of cell migration and wound closure in extract-treated cells. The extract exhibited 74.07% wound closure after 48 hours, indicating substantial wound healing activity when compared with the standard drug Cipladine, which showed 87.53% wound closure. These findings suggest that the extract promotes fibroblast migration, an essential step in tissue repair and regeneration.

The observed wound healing activity may be attributed to the synergistic action of the phytoconstituents present in the extract. Flavonoids and phenolic compounds are known to reduce oxidative stress, while tannins and terpenoids may support antimicrobial activity and tissue remodeling. Such combined effects can accelerate the wound healing process and improve cellular recovery.

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Final conclision are the study provides scientific evidence supporting the traditional medicinal value of *Jatropha integerrima*. The results indicate that the hydroalcoholic leaf extract possesses promising wound healing properties and may be considered a potential natural source for the development of herbal wound care formulations. Further studies involving isolation of active constituents, mechanistic investigations, and in vivo evaluations are recommended to validate its therapeutic efficacy and safety.

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