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ORIGINAL ARTICLE

Phytochemical Profiling and Experimental Validation of Wound and Burn Wound Healing Activity of the Whole Plant of *Euphorbia hirta* Linn

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Abstract
Background: Wound repair represents a highly intricate, multi-phase biological sequence encompassing hemostasis, inflammation, proliferation, as well as subsequent remodeling, while burn injuries further impede this progression due to the presence of tissue necrosis, intensified inflammatory responses and microbial infiltration. The rising challenge of antimicrobial resistance, along with escalating treatment expenses, has consequently prompted renewed attention toward plant-derived therapeutics, and Euphorbia hirta Linn., long utilized across diverse traditional medicinal systems, contains numerous bioactive constituents that exhibit considerable potential in facilitating wound healing. **Objectives:** To evaluate the effectiveness of the ethanolic extract derived from the entire *Euphorbia hirta* Linn. plant in promoting wound and burn healing is assessed through pharmacognostic and phytochemical approaches, along with the use of animal-based experimental models. **Methods:** Once collected and identified, the whole plant was subjected to Soxhlet extraction using ethanol and then followed by fractionation. Standard procedures were employed to perform pharmacognostic as well as physicochemical evaluations, while preliminary phytochemical screening along with TLC and spectroscopic analyses was carried out. Wound-healing activity was evaluated using excision, incision, dead space and burn wound models, during which parameters such as wound contraction, epithelialization period and tensile strength were recorded. **Results:** With physicochemical parameters remaining within acceptable limits, the quality of the plant material was thereby confirmed. Phytochemical screening revealed the presence of flavonoids, tannins, phenolics, saponins as well as glycosides, particularly in the ethanolic extract. In comparison to the control, the extract showed a significant increase in wound contraction (92 ± 1.2% on day 12), enhanced tensile strength (378 ± 14 g) and a shortened epithelialization period (12 ± 0.9 days. **Conclusion:** The ethanolic extract of *Euphorbia hirta* Linn. demonstrates significant wound as well as burn wound healing activity, likely attributed to the synergistic effects of its bioactive phytoconstituents. The study thereby provides scientific validation for its traditional use and underscores its potential as a cost-effective herbal alternative for wound management, particularly in resource-limited settings.

Keywords: Euphorbia hirta, wound healing, burn injury, phytochemicals, flavonoids, antioxidant

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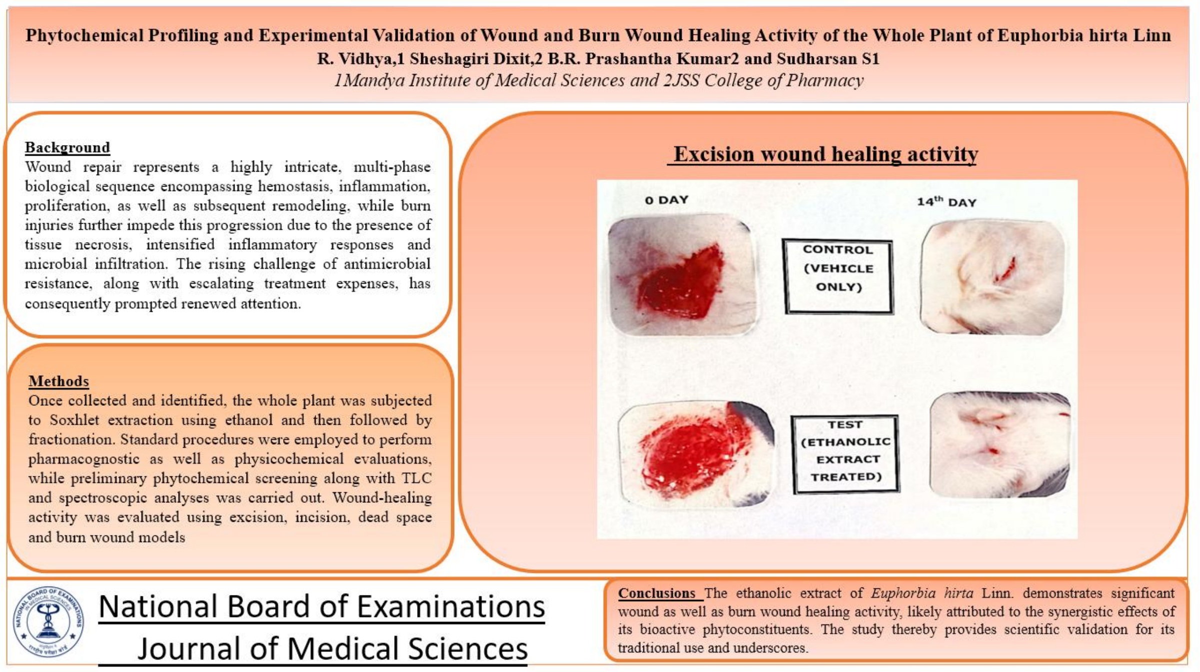
Graphical Abstract



Introduction

A tightly controlled and intricate biological sequence, wound healing entails coordinated interplay among diverse cell types along with extracellular matrix components, growth factors, cytokines and signaling molecules, thereby enabling restoration of tissue integrity following injury. Proceeding through overlapping stages—namely hemostasis, inflammation, proliferation and remodelling—the process initially limits excessive blood loss through hemostasis immediately after injury, after which the inflammatory phase, marked by infiltration of neutrophils and macrophages, functions to clear debris as well as prevent infection. Subsequently, the proliferative stage is characterized by fibroblast migration, angiogenesis, collagen synthesis and re-epithelialization, whereas the remodeling phase ultimately facilitates maturation and reorganization of collagen fibers, resulting in restored tensile strength [1,2].

Impairment of any of these phases can hinder the healing progression, with commonly observed causes including microbial infection, oxidative stress, ischemia, impaired perfusion, metabolic disorders along with prolonged inflammation, which may consequently result in delayed wound closure or chronic non-healing wounds. Characterized by persistent inflammation, chronic wounds also exhibit elevated protease activity, diminished responsiveness to growth factors and inadequate extracellular matrix formation, all of which contribute to poor clinical outcomes as well as an increased healthcare burden [3,4].

Representing a severe form of tissue trauma, burn injuries are associated with extensive tissue necrosis together with loss of skin barrier function, while also demonstrating microbial colonization and imbalance in fluid and electrolytes, alongside an exaggerated local as well as systemic inflammatory response. Such alterations can delay epithelialization and enhance scar formation, in addition to increasing the risk of secondary infections, and in severe cases, burn injuries may lead to systemic inflammatory response syndrome and multiorgan dysfunction, which further complicates wound management and delays recovery [5,6].

Although modern wound care techniques have advanced, complications remain common despite the use of advanced dressings, antibiotics and surgical interventions, and wound- as well as burn-related complications continue to be a major cause of morbidity and mortality worldwide, with the burden being greater in low- and middle-income countries where limited healthcare access, high treatment costs and poor wound hygiene act as important contributing factors, while increasing antimicrobial resistance further compromises effective wound management [7].

Growing antimicrobial resistance along with the high cost of synthetic drugs has prompted increased interest in medicinal plants, with adverse drug reactions also contributing to this shift, as these plants are utilized as alternative as well as complementary therapies for wound and burn management due to their easy availability, cost-effectiveness and cultural acceptability, while containing multiple bioactive constituents that may act synergistically to promote healing [8,9].

Traditional systems such as Ayurveda, Sidha and Unani describe the use of medicinal plants for the treatment of wounds, burns, ulcers and other skin diseases, and these systems generally favor the use of whole plants or crude extracts, which is considered to provide balanced therapeutic effects owing to the combined action of phytoconstituents [10].

Euphorbia hirta Linn. (family Euphorbiaceae), commonly referred to as the “asthma plant,” is widely distributed across tropical and subtropical regions of Asia, Africa and South America, and is traditionally employed for respiratory as well as gastrointestinal disorders, while also being used for skin infections, wounds, ulcers and burns, where it is applied in the form of paste, decoction or latex and utilized for cuts, boils along with inflammatory skin conditions in many communities [11–13].

Phytochemical investigations of Euphorbia hirta indicate the presence of flavonoids, tannins, phenolic compounds and saponins, which exhibit antimicrobial, antioxidant and anti-inflammatory activities along with wound healing properties, as flavonoids and phenolic compounds facilitate free radical scavenging and enhance collagen synthesis, whereas tannins promote wound contraction and epithelialization; however, comprehensive evaluation of the whole plant using multiple wound healing models remains limited, which constituted the basis for the present study [14–16].

Materials and Methods

Collection and Authentication of Plant Material

The whole plant of Euphorbia hirta Linn. was collected from in and around the Mandya region during the monsoon as well as winter seasons, which were selected since phytoconstituent concentration is reported to be optimal [17], while seasonal variation can influence the biosynthesis and accumulation of secondary metabolites in medicinal plants, thereby affecting their pharmacological activity; only healthy plants were chosen, with care taken to avoid diseased specimens, insect infestation and environmental contamination.

The collected plant material was subsequently authenticated by a qualified taxonomist, and a voucher specimen was deposited in the departmental herbarium for future reference [18], as authentication ensures accurate botanical identification while also supporting reproducibility of the study, which is essential for pharmacognostic standardization and future investigations.

Preparation of Extracts

The collected plant material of Euphorbia hirta was thoroughly washed with water to remove soil and debris, after which it was shade dried to preserve thermolabile constituents, as this method helps prevent degradation of heat-sensitive phytoconstituents; the dried material was then coarsely powdered using a mechanical grinder, thereby increasing the surface area for extraction, and subsequently subjected to Soxhlet extraction using ethanol as the solvent [19]. The resulting total alcoholic crude extract was further fractionated using petroleum ether, solvent ether, ethyl acetate, butanol and butanone, where the use of solvents with increasing polarity facilitates extraction of different phytoconstituents, and each extract was filtered and concentrated under reduced pressure using a rotary evaporator before being stored in airtight containers at low temperature to prevent degradation.

Pharmacognostic Evaluation

Macroscopic as well as microscopic evaluation was performed to establish pharmacognostic standards [20], wherein macroscopic analysis included assessment of size, shape, color, surface and texture of plant parts, while microscopic examination identified features such as trichomes, stomata, vascular bundles and cellular inclusions, which aid in identification and detection of adulteration. Physicochemical parameters including total ash, acid-insoluble ash, water-soluble ash, extractive values and loss on drying were determined using standard methods [21], as these parameters reflect purity and quality of the plant material and contribute to quality control.

Phytochemical Investigation

Preliminary phytochemical screening was conducted using standard qualitative tests [14,22] to identify various classes of phytoconstituents, revealing the presence of alkaloids, flavonoids, tannins, saponins, glycosides, phenolics and steroids, thereby providing basic insight into the chemical nature of the plant and enabling correlation of pharmacological activity with its constituents. Thin-layer chromatography was performed to confirm flavonoids as well as phenolic compounds and to examine the chemical profile of the extracts using suitable solvent systems and detecting reagents, while UV–visible and FT-IR spectroscopy were carried out for characterization of phytoconstituents, aiding in identification of functional groups and preliminary structural features [23].

Pharmacological Evaluation

Acute Toxicity Studies

Acute oral toxicity studies were carried out in accordance with OECD guidelines [24], during which animals were monitored for behavioral changes, signs of toxicity and mortality, and the ethanolic extract was found to be safe at the tested doses, thereby indicating safety and supporting further investigations.

Evaluation of Wound Healing Activity

Wound healing activity was assessed using excision, incision, dead space and burn wound models [25,26], which are standard experimental approaches, and the use of multiple models enables evaluation of different aspects of wound healing including wound contraction, collagen formation, tensile strength and tissue repair. Parameters such as wound contraction, epithelialization period, tensile strength, granuloma formation and histopathological changes were measured [27], as these indicators reflect the rate as well as quality of healing and provide evidence for the activity of the extract.

Anti-inflammatory and Haemostatic Activity

Anti-inflammatory activity was evaluated by assessing inhibition of inflammatory mediators [28], given that inflammation plays a critical role in the early phase of wound healing and regulation of excessive inflammation supports proper healing, while haemostatic activity was determined by measuring bleeding time and clotting time [29], since rapid hemostasis is essential for wound repair and facilitates early stabilization of the wound.

Biochemical Investigation

Biochemical parameters including total protein, carbohydrate and antioxidant enzymes were estimated [30], as these factors contribute to collagen synthesis, fibroblast activity and tissue regeneration, thereby supporting the wound healing effects observed in experimental models.

Results

According to Table 1, the physicochemical parameters of Euphorbia hirta were found to be within acceptable pharmacopoeial limits, indicating good quality as well as purity of the plant material, where the total ash value (7.8%) reflects total inorganic content, the low acid-insoluble ash value (1.9%) suggests minimal siliceous contamination, and the water-soluble ash value (3.2%) indicates the presence of water-soluble inorganic salts; moreover, the alcohol-soluble extractive value (22.7%) being higher than the water-soluble extractive value (18.4%) implies that a greater proportion of phytoconstituents are soluble in organic solvents, while the loss on drying (6.3%) denotes low moisture content, which is beneficial for preventing microbial growth and maintaining stability of the crude drug.

Table 1. Physicochemical Parameters of *Euphorbia hirta*

Parameter	**Value**
Total ash (%)	7.8
Acid-insoluble ash (%)	1.9
Water-soluble ash (%)	3.2
Alcohol-soluble extractive (%)	22.7
Water-soluble extractive (%)	18.4
Loss on drying (%)	6.3

According to Table 2, the percentage yield of extracts varied with solvent polarity, with the aqueous extract showing the highest yield (14.2%), followed by ethanolic (12.6%) and methanolic extract (10.9%), thereby indicating the predominance of polar constituents, whereas petroleum ether (3.4%) and chloroform (5.8%) extracts exhibited lower yields, suggesting fewer non-polar constituents, and these findings support the selection of the ethanolic extract for further study as it provides a good yield while extracting a wide range of bioactive compounds.

Table 2. Percentage Yield of Various Extracts

Solvent	Yield (%)
Petroleum ether	3.4
Solvent Ether	5.8
Ethyl Acetate	12.6
Butanol	10.9
Butanone	14.2

According to Table 3, preliminary phytochemical screening revealed the presence of diverse phytoconstituents, with ethanolic as well as aqueous extracts containing a greater number of bioactive compounds, where flavonoids and phenolic compounds were present in high amounts (+++) in the ethanolic extract, while tannins and saponins were more abundant in the aqueous extract, and glycosides were detected in both extracts; in contrast, the petroleum ether extract showed minimal phytochemical presence, indicating limited non-polar constituents, and these observations suggest that polar extracts possess greater therapeutic potential.

Table 3. Preliminary Phytochemical Screening

Phytoconstituent	Petroleum ether	Chloroform	Ethanol	Aqueous
Flavonoids	–	+	+++	++
Tannins	–	+	++	+++
Phenolics	–	+	+++	++
Saponins	–	–	++	+++
Glycosides	–	+	++	++

According to Table 4, the ethanolic extract demonstrated a significant increase in wound contraction compared to the control at all time points, with wound contraction reaching 41 ± 2.3% on day 4, which was higher than the control and comparable to the standard group, increasing to 71 ± 1.9% on day 8 and further to 92 ± 1.2% on day 12, thereby indicating that the ethanolic extract facilitates faster wound closure.

Table 4. Effect of Ethanolic Extract on Wound Contraction (%)

Day	**Control**	**Standard**	**Extract**
4	22 ± 2.1	35 ± 1.8	41 ± 2.3
8	45 ± 2.4	62 ± 2.0	71 ± 1.9
12	68 ± 1.7	85 ± 1.5	92 ± 1.2

According to Table 5, tensile strength in the incision wound model was higher in the extract-treated group (378 ± 14 g) compared to the control (210 ± 12 g) and was also slightly greater than the standard group (345 ± 15 g), suggesting enhanced collagen synthesis along with improved tissue maturation, where increased tensile strength reflects better quality of healing.

Table 5. Tensile Strength in Incision Wound Model

Group	**Tensile Strength (g)**
Control	210 ± 12
Standard	345 ± 15
Extract	378 ± 14

According to Table 6, the epithelialization period was reduced in the extract-treated group, with complete epithelialization occurring in 12 ± 0.9 days compared to 21 ± 1.3 days in the control group and 14 ± 1.1 days in the standard group, indicating faster epithelial regeneration and further supporting the wound healing activity of the ethanolic extract of *Euphorbia hirta.*

Table 6. Effect on Epithelialization Period

Group	Days for Complete Epithelialization
Control	21 ± 1.3
Standard	14 ± 1.1
Extract	12 ± 0.9

Figure 1 showing the macroscopic morphology of the whole plant of *Euphorbia hirta* Linn., highlighting the characteristic erect stem, opposite leaves and clustered inflorescences at the nodes. The image represents the plant material used for pharmacognostic evaluation, phytochemical investigation and experimental wound healing studies.

Figure 1. Whole plant of *Euphorbia hirta* Linn.

Figure 2 showing *Euphorbia hirta* Linn. growing in its natural habitat on dry, open soil. The image highlights the prostrate to semi-erect growth habit of the plant, opposite elliptic leaves and clustered inflorescences at the nodes, characteristic of the species. The photograph represents the natural ecological distribution of the plant from which the material used in the present study was collected.

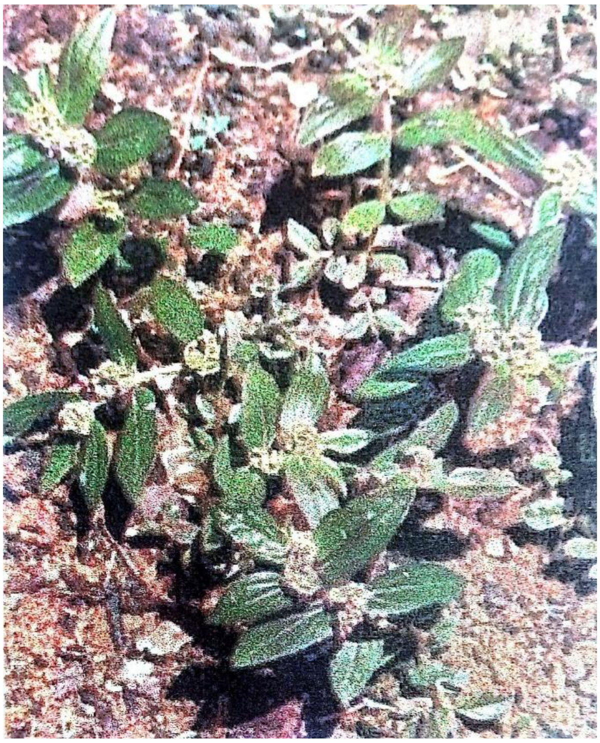


Figure 2. Natural habitat of *Euphorbia hirta* Linn.

Figure3 depicting the effect of ethanolic extract of *Euphorbia hirta* Linn. on excision wound healing in experimental animals. Images show wounds on **day 0** and **day 14** in the **control group (vehicle only)** and **test group (ethanolic extract treated)**. The test group demonstrates enhanced wound contraction and faster epithelialization compared to the control group, indicating significant wound healing activity of the ethanolic extract.

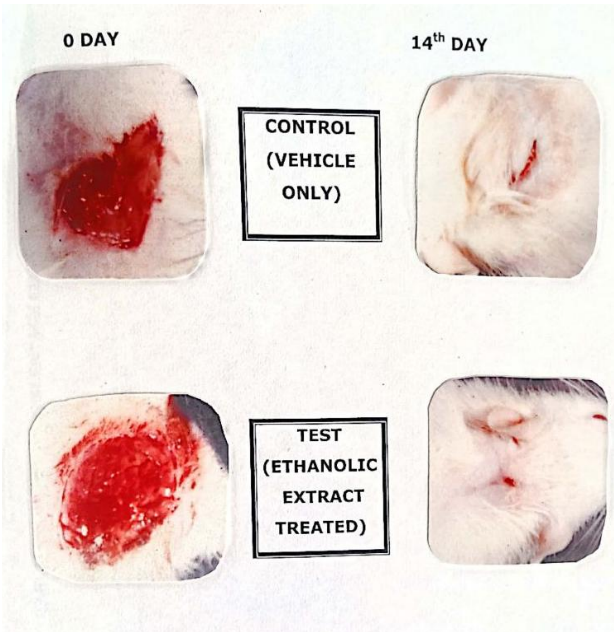


Figure 3. Excision wound healing activity

Discussion

The enhanced wound healing activity of the ethanolic extract of Euphorbia hirta may be attributed to the combined action of flavonoids, tannins and phenolic compounds present in the plant [14–16], where the presence of multiple phytoconstituents acting synergistically can produce a better therapeutic effect than isolated compounds, thereby supporting the use of whole plant extracts in traditional medicine; flavonoids facilitate fibroblast proliferation, collagen synthesis and angiogenesis, which are critical processes in the proliferative phase of wound healing, while also modulating growth factors and promoting re-epithelialization, leading to faster wound closure [15,22].

Tannins likewise contribute to wound healing by promoting wound contraction and epithelialization through their astringent action, as they induce precipitation of proteins and form a protective layer over the wound, thereby facilitating faster tissue repair while reducing the risk of infection and fluid loss from the wound [15,22], whereas phenolic compounds exert their effect through free radical scavenging and stabilization of the extracellular matrix, which enhances tissue strength and integrity [14–16].

The antimicrobial activity of the extract plays a crucial role in wound healing, since infection represents a major cause of delayed healing and chronic wounds [8,27], and microbial proliferation prolongs inflammation while impairing tissue regeneration; by inhibiting microorganisms, the extract helps maintain a clean wound environment, thereby supporting proper healing and reducing the risk of wound sepsis [8,27].

Antioxidant activity mitigates oxidative stress at the wound site, which promotes faster tissue regeneration and prevents cellular damage [28,30], as reactive oxygen species can damage cell membranes, proteins and nucleic acids, thereby delaying healing, whereas the antioxidant effect of Euphorbia hirta may protect newly formed tissue and facilitate the transition from the inflammatory to the proliferative phase [28,30].

Anti-inflammatory as well as haemostatic activities further support wound and burn healing, since appropriate regulation of inflammation is essential for effective healing while excessive inflammation can delay recovery and increase scarring, and haemostatic activity promotes early wound stabilization by reducing blood loss and facilitating clot formation, which serves as a matrix for cell migration and tissue repair, thereby collectively explaining the wound and burn healing effects observed in this study.

Conclusion

The present study offers scientific validation for the traditional use of Euphorbia hirta Linn. in the management of wounds as well as burn injuries, as the ethanolic extract of the whole plant demonstrated significant wound healing, burn wound healing, antimicrobial, antioxidant, anti-inflammatory and haemostatic activities, which were evidenced by increased wound contraction, reduced epithelialization period and enhanced tensile strength in experimental models, and these effects may be attributed to the combined action of bioactive phytoconstituents present in the plant.

Being widely distributed and readily available as a weed, Euphorbia hirta is also low in cost, and due to these advantages it exhibits considerable potential for development into a herbal wound care formulation, while the findings of this study support its traditional use and provide a foundation for further formulation development as well as clinical investigations; with appropriate standardization and safety evaluation, it may serve as a cost-effective alternative for wound and burn management, particularly in resource-limited settings.

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Statements and Declarations

Conflicts of interest

The authors declare that they do not have conflict of interest.

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