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Exploring the Wound Healing Potential of *Tridax procumbens* Extract: A Comprehensive Analysis through *in vitro* Cytotoxicity and Scratch Assay Studies

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ABSTRACT

Background: Wound healing, a complex biological process, is crucial for preventing infections and maintaining skin integrity. This comprehensive review examines wound dynamics based on location, etiology, symptoms, and clinical appearance. Emphasizing the importance of precise progression through inflammation, cellular proliferation, and remodeling phases, the study highlights the risk of chronic wounds with delays or interruptions.

Methods: This study explores natural alternatives to synthetic wound healing drugs, focusing on *T. procumbens*. Scientific investigations reveal its diverse pharmacological activities, particularly in ointment formulations. *In vitro* cytotoxicity and scratch tests, using the MTT test on Vero-E6 cell lines, demonstrate *T. procumbens*' potential for wound healing. The research aims to address the challenges posed by the side effects of synthetic drugs.

Results: The research provides insights into skin tissue regeneration through *T. procumbens*, revealing its ability for wound healing. The cytotoxicity index is calculated after applying drug concentrations, and regression analysis yields the IC50, offering information on the effect on cell viability.

Conclusion: Combining traditional wisdom with contemporary research, this study bridges the gap between technology and tradition, presenting a comprehensive method for wound management. *T. procumbens* emerges as a promising herbal treatment, with its potential demonstrated through diverse pharmacological activities and cellular reactions.

Key-words: Cellular proliferation, Chronic wounds, Cytotoxicity, Inflammation, Medicinal plants, Remodeling phases, *Tridax procumbens*, Wound healing

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INTRODUCTION

Wound healing is a sophisticated process involving tissue restoration following injury or damage. A wound is described as disrupting the normal continuity of the skin, leading to a break in its cellular and anatomical structures, thereby affecting its regular functionality. This intricate process can be classified based on the wound's location, origin, presenting symptoms, type of injury, depth, tissue loss, or clinical appearance. A comprehensive understanding of the multifaceted

nature of wounds is crucial for developing effective strategies for their management and promoting optimal healing outcomes.

The healing process can be conceptualized into three overlapping phases: inflammation, cellular proliferation, and remodelling. These phases are carefully orchestrated to ensure the restoration of normal tissue function [1]. During the initial inflammatory phase, the body responds to the injury by eliminating pathogens and cellular debris through the influx of cytokines and growth factors. Subsequently, the cellular proliferation phase involves the formation of granulation tissue through processes like angiogenesis, with keratinocytes and fibroblasts migrating to the wound site. The maturation phase is marked by restoring the skin barrier, repairing granulation tissue within the scar, and vessel regression [2]. This newly formed tissue gradually gains tensile strength, with collagen production, cross-linking, and reorganization occurring over an extended period [3]. The accurate and regular progression of these phases is crucial, as interruptions or prolongations can lead to chronic wounds or delayed healing, often associated with conditions such as ischemia, diabetes, and hypertension [4].

A wound disrupts cellular, anatomical, and functional continuity in living tissue resulting from various insults, including physical, chemical, thermal, microbial, or immunological insults. Essentially, a wound is a break in epithelial integrity accompanied by disruption in the structure and function of underlying normal tissue [5-8]. While the natural healing process of wounds initiates and progresses, the outcomes are not always favorable [9].

To enhance the quality and speed of the healing process, synthetic drugs like nitrofurazone, gentamicin, and mupirocin are commonly used, which have many side effects. In recent years, there has been a growing trend towards using natural medicines to mitigate these drawbacks [10]. Many medicinal plants, with a long history of efficacy in various diseases, have been explored for their potential in wound healing. However, despite their traditional use, there is often a lack of scientific data or information to prove their effectiveness, understand their active ingredients, or elucidate their mechanisms of action [11].

In this context, *T. procumbens* leaves emerge as one of the medicinal plants traditionally used for wound healing, particularly by tribal communities. *T. procumbens*, commonly known as "Coat buttons" or "Mexican daisy," is a weed plant belonging to the Asteraceae family. It is often found in waste places, roadsides, and hedges. The leaves of *T. procumbens* contain various components such as 26% crude protein, 17% crude fibre, 39% soluble carbohydrates, 5% calcium oxide, and 5% Luteolin, glucoluteolin, and quercetin. Traditionally, the matured leaves are crushed to make a paste and applied to the surface of wounds. Additionally, these leaves have been used for various ailments like high blood pressure, bronchial catarrh, dysentery, malaria, diarrhoea, and to prevent hair fall [12-14]. Scientific investigations into *T. procumbens* have revealed a wide range of pharmacological activities, including antioxidant, antimicrobial, anti-inflammatory, vasorelaxant, anti-leishmanial, anti-arthritic, antihypertensive, hepatoprotective, anticancer, hemostatic, anti anemic, and anti-diabetic properties [15].

Cell culture techniques are indispensable in developing biotechnology products, as they provide quick setups and a wide range of in vitro tests such as proliferation, migration, viability, inflammation, and apoptosis [11]. The comparison of cell migration outcomes using *different in vitro* wound healing techniques provides valuable insights into the intricate interplay between cellular responses and external factors [16]. The present study aims to harness the potential of *T. procumbens* extract for wound healing.

MATERIALS AND METHODS

Formulation of extract- *T. procumbens* plants were gathered from Misrod Bhopal farms to formulate the gel. To prepare the extract, the flowers from the collected plants were removed, and the remaining components were cleaned with tap water, distilled water, and filter paper under the sun for seven days. Following the entire drying of the plants, the powdered leaves stems, and roots were gathered and put through sieve No. 12 to eliminate any remaining debris. Following this, a 1:10 ratio of powder to water was used for dissolution. This mixture was incubated for a whole day. After that, it was filtered, and the filtrate was saved for the water to evaporate [17].

Cytotoxicity Test- To assess the potential toxicity of *T. procumbens* extract on cells. In brief, 5000 cells per well

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were seeded in 96-well plates, and the cells were exposed to the ointment for 24-72 hours, with each condition performed in triplicate. Cell growth was evaluated using MTT dye. 100 µL of MTT dye (0.5 mg working stock) was added to each well and incubated for 4 hours. Following incubation, the formazan crystals formed within the cells were solubilized using dimethyl sulfoxide, and the optical density was measured at 570 nm using a spectrophotometer [18]. The percentage of cell growth cells was determined using the following formula:

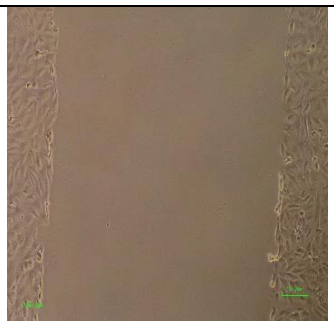
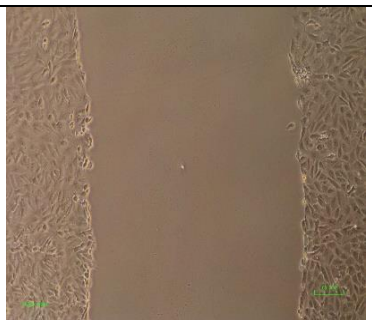
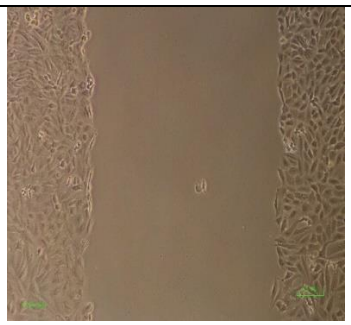
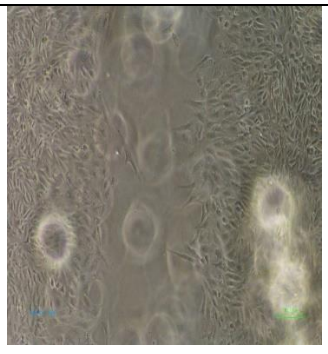
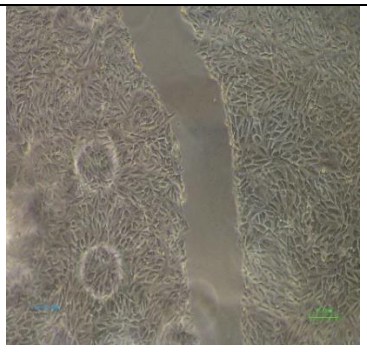
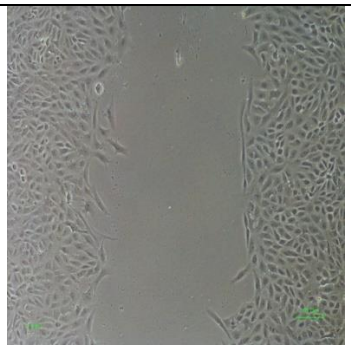
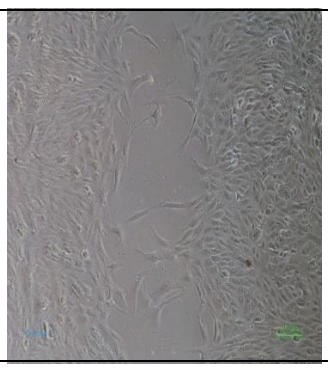
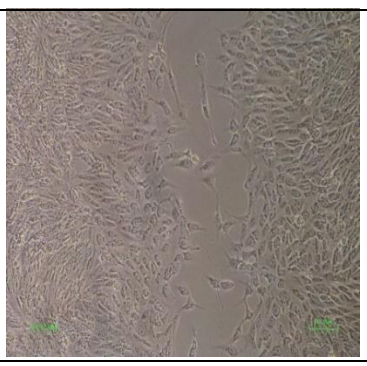
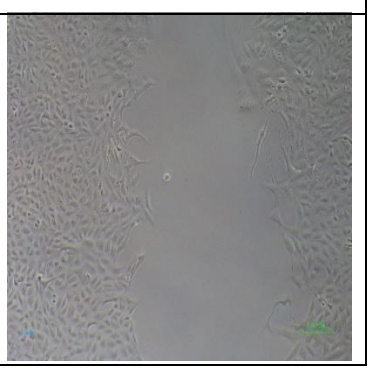
Cell growth (%) = A570/630 of treated cells / A570/630 of control cells

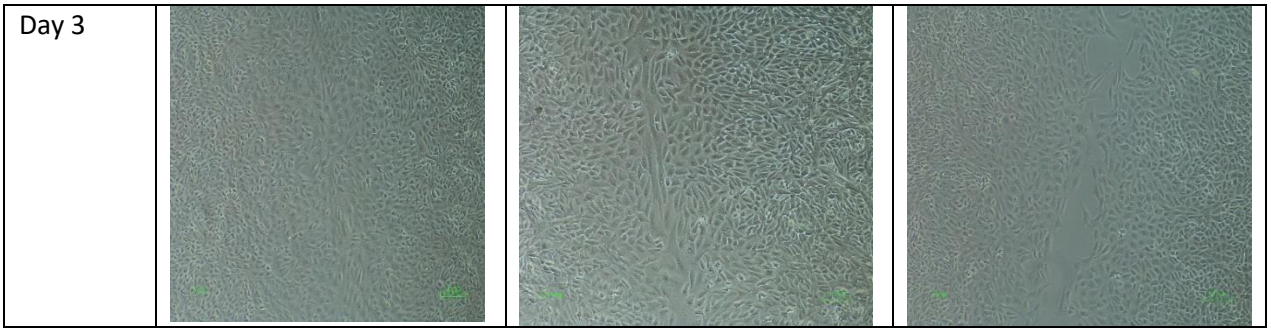
Scratch assay- To determine the effect of the compound on cell growth, scratch assay was performed. In the present study, 5000 cells/well were seeded in 96-well plates, and cells were treated with *T. procumbens* extract for 24-72 hours (in triplicate). Cell growth was measured using MTT dye and incubated for 4 hours. The percentage of cell growth cells will be determined using the formula: cell growth (%) = A570/630 of treated cells/A570/630 of control cells [18].

RESULTS

The drug-containing *T. procumbens* extract was dissolved in Acetone. The IC_{50} was calculated using MTT assay. The range of IC_{50} was 110µg to 125.5 µg. Accordingly, two different concentrations of the drug were used 4µg/µl & 10µg/µl. 10µl of both concentrations were added to 24-well plate. The final drug concentration in each well was 40µg & 100 µg respectively. The result of the scratch assay depicted the effectiveness of both concentrations. But, the 40µg drug concentration was found to be more effective (Table 1).

Table 1: Cell migration appeared between scratch area in Vero E 6 Cell

Intervals	Concentration of Ointment (Vero E6 cell line)		Control Vero E6 cell line
	40 ug (10ug/ul)	100 ug (10ug/ul)	
Day 0			
Day 1			
Day 2			



Day 0: Scratch was introduced in vero cell line and after that T. procumbens extract was inoculated in the cell lines, Day1: Cell synthesis was started, Day 2: Cell were come closer Day 3: Wound was completely closed.

DISCUSSION

In the wound healing process, cell migration plays a crucial role in promoting wound closure. The quantification of wound closure following various treatments is illustrated in Table 1. In the present study, vero cells exhibited 99.9% migration toward the scratched wound after 24 hours. It is crucial to note that cell migration is a pivotal event in wound healing, triggering subsequent stages of the curing process. Therefore, investigating vero cells migration after exposure to drug formulations is essential for developing new therapies in wound healing. The scratch assay, a well-established in vitro method for measuring cell migration, assessed the wound-healing efficacy of *T. procumbens* leaf extract.

Numerous studies have been conducted on wound care, emphasizing cutting-edge therapeutic strategies and the creation of plant-based products for treating various wound types. In the excision wound model, Benito *et al.* [19] conducted a study wherein animals were categorized into two groups, each consisting of six animals. The groups were as follows: Group-I (Control), Group-II (Nimesulide), and Group-III (Ethanol extract of *T. procumbens*). Their investigation revealed that administering *T. procumbens* extract, even at doses up to 2000 mg/kg body weight, did not elicit any toxic symptoms or mortality.

No changes in behavioral patterns or indications of toxicity were noted during the 24-hour observation period. As a result, it can be inferred that the extract is safe for subsequent pharmacological evaluations.

The wound scratch assay demonstrated a cell migration rate of approximately 90±2% during a 72-hour incubation period, in contrast to the control group, which showed a rate of 62±2%. This outcome indicates a notable enhancement in cell migration facilitated by the herb ointment, leading to the complete disappearance of the scratch area within three days. This accelerated cell migration suggests a potential stimulation of epithelization, proliferation, and collagen viability near the wound region [20].

The ointment formulated with an ethanolic extract of *T. procumbens* demonstrates noteworthy wound healing efficacy, particularly in facilitating wound contraction, especially at a 100 mg/g concentration. However, when compared at the same concentration, standard betadine exhibits higher wound contraction than the test ointment [21].

The ability of *T. procumbens* to cure wounds has been examined in this study. It was found that the *T. procumbens* extract enhanced wound closure in Vero cell lines. Moreover, it was discovered that the extract exhibited no cytotoxic properties. The results of this study suggest that *T. procumbens* could heal wounds and be a good place to locate natural compounds that can do the same. Talekar *et al.* [22] reported that, at a concentration of 20 mg/ml formulation, a notable increase in tertiary and quaternary vessels was observed, attributed to the angiogenic potential of the formulation. The formulation at concentrations of 3 mg/ml and 5 mg/ml demonstrated significant mobilization of keratinocytes and fibroblasts at the injury site. The findings of our current study align with the research conducted by Venkatachalam and Palaniswamy [23]. They observed that the extract of *T. procumbens* leaf demonstrated significant potential as a wound healing agent. Their results indicated a notable reduction in the scratch open area, leading to nearly complete closure of scratches (94%) within 24 hours. Importantly, this effect was achieved without inducing cytotoxicity in L929 mouse fibroblast cells. The difference between the present investigation and Venkatachalam and

Palaniswamy [19] was that they studied L929 mouse fibroblasts and in the present study, excellent wound healing properties in vero cells was observed. Previous studies reported that the polyherbal formulation exhibited prompt skin regeneration and wound contraction.

Regarding biochemical parameters, there was an elevation in hydroxyproline, hexosamine, and collagen turnover in animals treated with the test drug compared to untreated animals [22]. In subsequent studies, it is imperative to conduct further tests adhering to ethical guidelines. These investigations aim to identify optimal treatments for healing wounds and bed sores in diabetic patients.

CONCLUSIONS

The study highlighting *T. procumbens's* efficacy in promoting wound closure and its non-toxic nature offers a promising avenue for advanced wound care, especially for diabetic patients. Formulating an ointment from its ethanolic extract could significantly benefit diabetic wound management by harnessing the plant's healing properties. Future steps include conducting specific clinical trials on diabetic wounds to confirm efficacy and safety, elucidating the mechanisms through which the plant aids wound healing, and optimizing the formulation for consistent potency. Regulatory approval will be crucial for market introduction, and educating healthcare professionals and patients about this novel treatment option. Additionally, exploring the potential for synergistic effects with other wound-healing agents could lead to even more effective treatments. This approach not only aims to improve healing outcomes for diabetic wounds but also opens up new possibilities for natural, plant-based wound care solutions, potentially transforming the landscape of wound management for diabetic patients and beyond.

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