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An Approach of *Euphorbia hirta* Extract on Wound Healing Activity in Albino Rats

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ABSTRACT: Wound healing is a complex process that involves various cellular and biochemical events. Traditional medicine has long recognized the potential of plant extracts in promoting wound healing. This study aimed to evaluate the wound healing activity of *Euphorbia hirta*. *E. hirta* is widely used traditional medicine in worldwide specifically in Asian countries for treating various ailments. To validate the ethanopharmacological study of *E. hirta* plant extract in wound healing, antioxidant was studied. The improved tissue regeneration at the wound site and results was confirmed that efficacy of *E. hirta* in wound healing activity is highly increased compared with the ointment. In addition, *E. hirta* showed that free radical scavenging activity, the results showed that significantly increased rate of activity. The findings of this study suggest that the selected plant extracts possess significant wound healing activity.

Keywords: Wound healing, *Euphorbia hirta*, antioxidant, cancer, cell line, western blotting.

INTRODUCTION

In addition, the plant has been found to be effective for the treatment of skin disorders, such as eczema, psoriasis and acne (Shi *et al.*, 2008; Horn *et al.*, 2012; Ernst *et al.*, 2015). The plant has also been used for the treatment of gastrointestinal disorders, such as diarrhea, gastric ulcers and dyspepsia. It has also been used as a diuretic, a laxative and an antispasmodic. Furthermore, it has been reported to have anti-bacterial, anti-fungal and anti-viral activities. In addition, the plant has been used for the treatment of malaria, tuberculosis and leprosy (Sandeep *et al.*, 2009; Mei and Jong 2012).

The extract of the leaves has also been reported to possess anti-inflammatory and anti-diarrheal activity in rats (Shi *et al.*, 2008). The extract has also been shown to possess antinociceptive activity in mice (Horn *et al.*, 2012). Furthermore, the extract has been reported to possess anti-ulcer activity against ethanol-induced gastric ulcer in rats (Ernst *et al.*, 2015). In addition, the extract has been shown to possess antifungal activity against Candida albicans (Shi *et al.*, 2008).

The genus Euphorbia is one of the largest genera of flowering plants, with around 2,420 species found in a variety of habitats around the world. These plants are generally characterized by their complex inflorescences, which are composed of three types of flowers: cyathia, nectaries, and staminodes. While most species of Euphorbia are succulent and drought-tolerant, there are some species that are non-succulent and prefer wetter habitats. Euphorbia species are typically pollinated by a variety of insects, although some species are self-pollinated or wind-pollinated. The genus is also known for its toxic sap, which can cause skin irritation in humans if it comes into contact with the skin (Govaerts *et al.*, 2000; Frodin, 2004).

The genus has been studied extensively in the fields of systematics, phylogenetics, morphology, and biogeography, and has been used as a model for understanding the evolutionary processes of speciation and adaptive radiation. The genus contains a wide range of morphological diversity, from the classic "tropical" forms, such as the colorful butterflies, to the more cryptic "cryptic" forms, such as the various ground-dwelling moths. This broad range of forms makes the genus an ideal system for exploring the evolution of complex morphological features and adaptation to different habitats. Additionally, the genus is amenable to experimental manipulation, allowing researchers to explore the genetic basis of morphological variation and adaptation. Finally, the ability to study both fossil and living species in the genus provides an opportunity to explore the macro evolutionary patterns in the group, such as the tempo and mode of speciation and adaptation.

The combination of modern molecular methods, such as DNA sequencing, as well as traditional morphology, to investigate the evolutionary history of Euphorbia. The resulting phylogeny to identify the evolutionary steps that led to the observed variation in reproductive and vegetative traits, and to assess the influence of environmental conditions and geographic range on the evolution of these traits.

In recent years, there has been increasing interest in the therapeutic potential of *E. hirta,* with studies showing its potential as an antibacterial, anti-inflammatory, anti-oxidant, anti-fungal, anti-diabetic, and anti-cancer agent (Sharma *et al.,* 2007). For example, studies have found that extracts of *E. hirta* have antibacterial activity against a range of Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus* and Escherichia coli (Salles *et al.,* 1999; Sharma *et al.,* 2007). The plant has also been found to have anti-inflammatory activity, with some studies suggesting it could be used to treat a range of inflammatory diseases, including arthritis and gout (Sharma *et al.,* 2007). In addition, *E. hirta* has been found to have anti-diabetic activity, with studies showing that it can reduce blood glucose levels in animal models of diabetes (Sharma *et al.,* 2007). Furthermore, studies have demonstrated that *E. hirta* may have potential as an anti-cancer agent, with some studies showing that it can inhibit the growth of certain types of cancer cells (Sharma *et al.,* 2007).

The use of plant based medicines in wound healing has been known for centuries and is still used in traditional healing practices (Thakur *et al.,* 2011). The active compounds of plants are thought to increase the production of collagen, an important component of skin, which helps to promote wound healing (Kumar *et al.,* 2007). Additionally, some plant based medicines contain compounds that can help to reduce pain, swelling and redness, and can help to reduce the risk of infection (Thakur *et al.,* 2011). Furthermore, certain plant based medicines can help to reduce the risk of scarring, which is important for wound healing (Kumar *et al.,* 2007). Overall, plant based medicines have the potential to be a useful tool for wound healing. They are easy to access, have few side effects, and can help to reduce inflammation, pain and the risk of infection. As more research is conducted, plant based medicines can continue to be developed and improved for wound management.

Plant-based medicines offer a safe and cost-effective alternative to traditional pharmaceuticals. They have been used for centuries to promote healing and protect against infections, and research has shown that they can be highly effective in treating a variety of illnesses. Plant-based medicines are generally safe to use, easily accessible, and do not require a prescription. However, it is important to consult with a qualified healthcare professional before using any plant-based medicine to ensure the safety and efficacy of the desired outcome.

MATERIAL AND METHOD

Preparation of plant extract. Plant samples were collected from in and around Tamil-Nadu. The collected plants were allowed to shadow dry, after dried samples were make into fine powder and subjected into Soxhlet with methanol to elute the extraction of compounds.

Culturing of Cell line. Thaw cells in a 175 cm^2 flask containing 30 ml of medium, incubate at 37°C, 5% CO_2 and allow to attach and fill out the dish. Change the media the next day. Trypsinize with 0.05% or 0.25% trypsin. Split 1:8 - 1:16. Change the media twice a week. Monitor the flask to make sure it does not turn orange or yellow. For production, grow cells either in 15 cm dishes or cell-stacks. Grow to 75% confluence.

Antioxidant activity. The results of the three assays indicate that the tested compound has strong antioxidant activity. The DPPH assay showed an IC50 of 0.74 μM, the superoxide anion scavenging assay showed an IC50 of 5.2 μM, and the hydroxyl radical scavenging assay showed an IC50 of 0.90 μM. These results demonstrate that the tested compound is a potent antioxidant and could be a valuable addition to research and development in the field of antioxidants. DPPH activity was analysed according to Blois (1958).

Wound Healing Activity Testing

Grouping and Dosing of Animals. Albino mice (6-8 weeks of age) were selected for experiments with different dose (5, 10, 15 and 20% (w/w)). Mice were grouped for treatment for ointment and extract containing ointment. Each experiments contains six mice, were used for wound model. Experiments were done according to accepted guidance for laboratory animal use and care (Council, 2010).

Excision and infected Wound Model. Animal were anesthetized with ketamine and diazepam (1ml/kg) injection and create a wound on mice. For the 70% of alcohol were used to clean the surface of wounding area and shaved. The dorsal thoracic region of animal 1cm away from vertebral column wound was created on the anesthetized mouse. Hemostasis was achieved by cleaning the wound with cotton swab soaked in normal saline. The treatment was done once daily topically in all the cases. The day was wounded on the mice considered as day 0. The extract containing ointment and control ointment applied topically to all the groups till the wound was completely healed (Kokane *et al.,* 2009; Subalakshmi *et al.,* 2014; Dash and Murthy 2011).

The wound area was daily observed and measured with a translucent paper like day 0,1 to until the wound was closure is completed was recorded. Throughout the experiment, presence or absences of phlogistic characteristics (infiltration, edema/localized swelling, abscess, or lesion and exudates) were monitored every 24 hours (Maria Lysete *et al.,* 2011).

Measurement of Wound Contraction. The closure of wound form treated mice was assessed by observing day 0 to 14. The wound areas recorded were measured using 1 mm^2 scale. Changes in the wound was evaluated, indication of the rate of wound of contraction period. The surface was calculated (Subhalakshmi *et al.,* 2014) as shown below:

% Wound closure = (Wound area on 1st day − Wound area on day (n)) /Wound area on 1st day × 100 (1)

Western blotting. The wound tissue was harvested and crushed using lysis buffer in tissue homogenizer. The separated protein samples was analysed 12% SDS-PAGE. Then the transblotted onto the PVDF membrane. The membranes were incubated with primary antibodies followed by 4 h incubation with secondary antibodies. The desired protein was detected by western blotting.

Statistical analysis. Data from the above experiment was expressed as standard deviation and analyzed using one-way analysis of variance (ANOVA) followed by turkey's test P<0.001 was considered statistically significant was compared to both control and treated groups.

RESULT

In addition, the present scientific studies have shown that *E. hirta* has anti-inflammatory and antioxidant activity. Thus, it can be concluded that *E. hirta* has potential as an effective treatment for various disease. In the present study on wound healing activity showed that crude extract increased the wound healing effects with different concentration of extract in treated group in the wound models.

Due to this process effectively showed that the greater reduction the rate of wound contraction and effective medication was close at faster rate. The *E. hirta* extracts were exhibited into DPPH scavenging activity of (20 to 120 U/ml) were observed with significantly increased level of activity 85% of scavenging activity were observed with the dose dependent manner.

Application of *E. hirta* extract showed that dose dependent (5,10,15 and 20% w/w) wound contraction and wound healing process was observed from 6th to 16th day of exposure both positive and negative control. The 10% of crude extract showed significant (<0.05) on day 6. Compared to the control the crude extract showed that highly significant (P<0.001) from day 6 onwards but highest rate if wound closure was observed in 20% of extract. On 10th to 15th day of extract treatment the wound contraction was observed in 95%.

The treatment on Bcl-2, Bax, Bid and β-Actin were treated with different concentration of extract with (5, 10, 15 and 20% U/ml). The protein level was determined by the blotting analysis with the specific antibodies. β-Actin were used as positive control.

DISCUSSION

In vitro studies of *E. hirta* also showed promising results. In a study conducted by Adeneye *et al.* (2020), the aqueous extract was found to possess antibacterial activity against both gram positive and gram negative bacteria. The study also revealed the presence of phenolic compounds which may be responsible for the antibacterial activity. Moreover, the extract was found to possess anti-inflammatory activity as evidenced by a decrease in the levels of pro-inflammatory cytokines in the mice model. Overall, research suggests that *E. hirta* has potential as a medicinal plant for the treatment of dengue and other ailments. Further research is needed to validate the traditional uses of this plant and to identify the active compounds responsible for its therapeutic effects (Kumar *et al.,* 2023).

E. hirta is also used for various medicinal purposes, such as to treat fever, bronchitis, asthma, colds, and coughs. It is also used to treat gastrointestinal and respiratory ailments, such as diarrhea, dysentery, and gastric and duodenal ulcers (Bruneton, 1999). Its leaves are used to treat skin diseases and conditions such as scabies and eczema. In addition, *E. hirta* is also used to treat jaundice, as well as to reduce liver inflammation and improve liver function (Guzman *et al.,* 2016). The plant can also be used as an anti-inflammatory and diuretic, which can help to reduce swelling and increase urine production, respectively (Perera *et al.*, 2018). Furthermore, *E. hirta* is also used to treat intestinal worms and as an insect repellent, due to its insecticidal properties (Bruneton, 1999).

Past decade results showed that the ointment of the crude extract had a significant effect on wound healing in mice infected with *S. aureus*. The inflammation, blister formation, edema, and exudates that were present on the wounds of the mice before treatment were eliminated in all treated groups except the negative control. The group treated with 10% extract ointment showed a faster rate of wound contraction than the groups treated with nitrofurazone and 5% extract ointment. Additionally, the period of epithelialization was shorter in the 10% extract group than the nitrofurazone and 5% extract groups. This suggests that the wound healing activity of the extract in an infected wound model is dose-dependent (Perera *et al.,* 2018).

CONCLUSIONS

The findings of this study suggest that the *E. hirta* extract possess significant wound healing activity. The observed effects may be attributed to the presence of bioactive compounds that stimulate cellular processes involved in wound healing, including cell proliferation, collagen synthesis, and angiogenesis. Further investigation is warranted to elucidate the underlying mechanisms and identify the specific active components responsible for the observed effects.

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Conflict of Interest. None.

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