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Angiogenic Potential of *Tridax procumbens* Leaves Extract: Insights from a Chicken Embryo Chorioallantoic Membrane (CAM) Model

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Abstract:

This study explores the angiogenic potential of *Tridax procumbens* leaves extract using the Chicken Embryo Chorioallantoic Membrane (CAM) model. Wounds, defined as damage to living tissue affecting skin epithelial integrity, initiate a complex wound healing process involving intricate cellular and tissue layer replacements. Angiogenesis, the formation of new blood vessels, is crucial in this process. Ointments, including herbal formulations, are applied externally for skin treatments. *Tridax procumbens* , known for its antibacterial and anti-inflammatory properties, is investigated for its angiogenic impact using the CAM model. The study involves meticulous standardization, dosage-dependent analysis, and the focal application method to assess angiogenic potential systematically.

Keywords: *Tridax procumbens*, angiogenesis, wound healing, CAM model, ointment, herbal medicine.

Introduction

A wound can be defined as damage to living tissue affecting the epithelial integrity of the skin's upper layer. As explained by the Wound Healing Society (WHS), wounds can result in the opening or breakdown of the skin system, causing disruptions in the anatomy, physiology, and function of the skin. The process of wound healing is intricate and complex, involving the replacement of devitalized and missing cellular structures and tissue layers. Wounds are broadly categorized into open and closed wounds. The mechanism of wound healingaimstorestorethenormalstructureandfunctionoftheskinandtissue.Essentially,wound healing is

a physiological process wherein damaged tissue is replaced by living tissue, leading to the restoration of tissue or skin integrity.

The mechanism of wound repair unfolds through various processes, including hemostasis, the inflammatory phase the proliferative or fibroblasts phase (encompassing collagen synthesis, neovascular formation, and re-epithelialization), and wound contraction, also known as tissue remodeling. This intricate series of events reflects the dynamic nature of the healing process and underscores the importance of understanding the physiological intricacies involved in restoring tissue integrity after injury (Shrivastava et al., 2020).

Angiogenesis, the process of forming new blood vessels from pre-existing ones, plays a crucial role in both growth and the metastasis of tumors, as highlighted by Tufan and Tufan (2005). The significance of angiogenesis is further underscored by the substantial health burden posed by both acute and chronic wounds. If left untreated or not spontaneously healed, an acute wound has the potential to evolve into a chronic condition, as discussed by Langer and Rajagopalan (2012). In addressing wounds, various treatment options are available, including moist dressings, autologous split-thickness skin grafts, surgical debridement, and the application of topical growth factors, each with its distinct advantages and limitations (Gupta et al., 2022).

Angiogenesis, beyond its involvement in tumor growth, is a pivotal mechanism controlling various physiological processes, such as embryonic development, the menstrual cycle, wound healing, and tissue repair following surgery or traumatic injury (Carmeliet, 2003). This intricate process is finely regulated by both stimulators and inhibitors. Any imbalance in these regulatory factors is linked to diseases such ascancer, cardio-cerebral diseases, age-related macular degeneration, arthritis, psoriasis, obesity, and asthma. Consequently, the modulation of angiogenesis is seen as an appealing and optimistic strategy for treating these pathological conditions. Understanding and manipulating angiogenic processes offer promising avenues for therapeutic interventions in a range of health conditions associated with disrupted blood vessel formation.

Ointments, characterized by their semisolid form, are externally applied for various skin treatments, such as burns and wounds (Thalkari et al., 2020; Laurent et al., 2023). While many commercial antibiotic ointments are commonly used for both dry and moist epidermal wounds, their efficacy may be compromised due to the potential for excessive bacterial growth on damaged skin layers, as noted by Kirsner and Eaglstein (1993)and Krysiak and Stachewicz (2023). Additionally, the application of topical antibiotic ointments may be limited by the risk of causing allergic reactions.

Generally, ointments exhibit a greasy or oily consistency and often consist of water-in-oil (W/O) emulsion (Agrawal et al., 2010; Chauhan and Gupta, 2020). Herbal medicines or plant-derived chemicals are recognized for their potential to influence the angiogenesis process (Loboda et al., 2005). They may exhibit either anti-angiogenic properties, as observed in studies by Hoseinkhani etal.(2020), Kooti etal .(2017),and

Lu et al. (2016), or promote angiogenesis, as indicated by Gupta et al. (2020). It is crucial to identify these activities before incorporating herbal preparations into specific applications.

Angiogenesis is a vital mechanism in the wound healing process, contributing to the formation of new blood vessels. Recognizing this, many herbal preparations are empirically employed for wound healing purposes. However, the quality, safety, and efficacy of alternative and complementary medicine, including herbal remedies, should be thoroughly tested before widespread use in the broader population, as emphasized bySeo et al. (2013). This cautious approach ensures that these alternative treatments are reliable and safe for promoting optimal wound healing outcomes.

Tridax procumbens, an herbal plant belonging to the Asteraceae family, has been identified as a rich source of chemical compounds, including flavonoid procumbenetin, sterols, polysaccharides, pentacyclictri terpenes, alkyl esters, and fatty acids, as highlighted by Gubbiveeranna and Nagaraju (2016) and Bhagat andKondawar (2019). Furthermore, the plant is endowed with essential minerals such as sodium, zinc, copper, manganese, iron, and various traceelements, includingphosphorous, calcium, potassium, andmagnesium, as elucidated by Oguntibeju (2019).

Numerous studies have underscored the extensive utilization of *Tridax procumbens* in traditional medicine, attributed to its antibacterial, antifungal, and anti-inflammatory properties. Additionally, the plant exhibits topical dermal wound healing capabilities against pathogens affecting the wound site, as noted byKubendiran et al. (2021), Baile and Parmar (2023), and Muthukumar et al. (2023).

To further elucidate the potential benefits and contribute to existing literature, a study has been initiated to evaluate the angiogenic potential of *Tridax procumbens* leaves extract using the chicken embryo chorioallantoic membrane (CAM) model. Herbal medicines, particularly those derived from *Tridax procumbens* , have been proposed to exert influence on angiogenesis, either by promoting or inhibiting the process. This specific investigation is focused on assessing the angiogenic potential of the *Tridax procumbens* leaves extract through the CAM model. The primary objective of the study is to systematically evaluate the angiogenic potential of the plant extract from *Tridax procumbens* Schrad. This research contributes valuable insights into the potential therapeutic effects of *Tridax procumbens* , specifically its impact on blood vessel formation, which is a crucial aspect of wound healing and tissue repair.

Materialsand methods:

CollectionofMedicinalPlantsandExtractionofAntimicrobialCompounds

The leaves of *Tridax procumbens were* gathered from the farm lands of Misrod, Bhopal, India. After transportation to the laboratory for identification, a specimen was stored for future studies. The leaves were shade-dried for ten days and then powdered using a mixer grinder for phytochemical extraction.

Formulation of Natural Herbal Medicated ointment

Beeswax and olive oil served as the base for the ointment, the herbal oil infusion and beeswax mixture were heated up to 60°C on a hot plate until the wax was completely melted. The warm mixture was then poured into balm containers.

Chicken embryo chorioallantoic membrane (CAM)assay:

The Chicken embryo chorioallantoic membrane (CAM) model was chosen as a suitable method for evaluating angiogenic potential due to its high vascularization and easy accessibility, as noted by Ribatti (2013).It'sworthmentioningthatexperimentsconductedonchickenembryosdonotrequireapprovalfrom an institutional animal ethics committee.

To ensure the reliability and reproducibility of the CAM model in laboratory conditions, a standardization process was undertaken. This involved continuous monitoring of the incubation temperature, maintaining humidity in the incubator at 96%, and rotating the eggs every 2 hours during the initial eight days of incubation. The successful standardization was confirmed by the hatching of chick embryos at the culmination of the 21st day of embryonic development, as validated by West et al. (2001).

In the assessment of the angiogenic potential of *Tridax procumbens* extract, the Whatman filter paper disc method, a focal application approach, was employed. The study of angiogenic potential was conducted in a dose-dependent manner using both methods. This robust experimental design ensures the systematic and controlled examination of *Tridax procumbens* extract, providing valuable insights into its impact on blood vessel formation within the CAM model. The utilization of the CAM model, coupled with meticulous standardization and dosage-dependent analysis, enhances the precision and reliability of the study, contributing to a comprehensive understanding of *Tridax procumbens* 'angiogenic potential.

The focal application method involved a series of precise steps to evaluate the angiogenic potential of *Tridax procumbens* leaves extract. The eggs, initiallycleaned with 70% ethanol, had a window created on the broad end on the third embryo development day (EDD), covered with micropore Scotch tape. This ensuredan aseptic condition throughout the procedure, conducted in a humidified incubator at 37 °C. Whatman filter paper discs of 2-mm diameter were autoclaved, loaded with 20 µl of the sample, and allowed to dry on a Perspex base.

Upon reaching the eighth EDD, the filter paper disc containing the *Tridax procumbens* leaf extract was strategically placed on the chorioallantoic membrane. This was achieved by puncturing a small hole in the membrane using a stainless steel dental needle size G24 in an area devoid of blood vessels. Photographic documentation of the application areas was performed at the time of sample application, and the eggs were left undisturbed for three days, with any observed mortality leading to the discard of affected eggs.

At the conclusion of the eleventh EDD, three days post-focal application, the chorioallantoic membrane was examined for angiogenesis, and photographs were taken for evaluation. The angiogenic potential of *Tridax procumbens* leaves extract was tested at concentrations of 85, 170, 255, 340, and 425 µg/disc. The assessmentinvolvedcountingthemeannumberofnewbloodvesselsaroundthedisc,and a classical

Angiogenic effect was defined by the spoke wheel appearance of blood vessels around the disc, as detailed by Vargas et.al.(2017).This meticulous procedure allowed for a systematic analysis of the angiogenic impact of *Tridax procumbens* leaves extract at various concentrations, providing valuable insights into its potential therapeutic effects on blood vessel formation.

Results and Discussion:

Results from the Chicken embryo chorioallantoic membrane (CAM) model revealed a notable increase in the mean number of blood vessels following the application of *Tridax procumbens ointment*. The examination of blood vessels on the membrane using the focal application method included three groups: the control group, paper disc containing *Tridax procumbens* onitment, and disc containing Plermin gel, a positive control known for promoting angiogenesis. Notably, the blank discs did not exhibit any growth in blood vessels. Maximum blood vessel growth was observed at a concentration of 150 mcg of *Tridax procumbens* leaves extract. In comparison, the Plermin control groups also demonstrated an increase in blood vessel growth, with the growth of vessels at 60 mcg of Plermin being equivalent to that observed with*Tridax procumbens* at 150 mcg.

Furthermore, visual changes in the growth pattern of blood vessels, characterized by an increase in number and supported by photographic evidence, were evident with the application of *Tridax procumbens ointment*.

In summary, the results highlight the positive impact of *Tridax procumbens leaves* extract on angiogenesis, with a concentration of 150 mcg showing maximum blood vessel growth. The visual changes observed and histological confirmation further strengthens the evidence of *Tridax procumbens* 'angiogenic potential. The parallel effects between *Tridax procumbens and* Plermin gel, a known angiogenic agent, underscore the promising therapeutic potential of *Tridax procumbens* in promoting blood vessel formation.

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