

## Research Article

### **Anti-inflammatory effect of rain tree leaf extract gel (*Samanea saman*) on fibronectin expression: study experimental**

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#### ABSTRACT

**Introduction:** A traumatic ulcer is an open wound on the skin or mucous membranes that often results from trauma, such as scratches, bites, shocks, or pressure. Rain tree leaves (*Samanea saman*) have the potential to reduce pain and anti-inflammatory effects thanks to the flavonoid content. This extract works by inhibiting the cyclooxygenase and lipoxygenase pathways, which help limit inflammation and accelerate wound healing. This study aims to evaluate the effects of a 12% concentration rain tree leaf (*Samanea saman*) extract gel on fibronectin expression in the process of healing traumatic ulcers. **Methods:** This experimental laboratory study employed a post test only control group design. The sample consisted of male white rat wistar *Rattus norvegicus* and a gel of rain tree leaf extract extracted with a 98% ethanol solvent using a maceration method with a 12% concentration. Data analysis was performed using the Mann-Whitney U test. **Results:** The highest average was observed in the treatment group receiving the 12% rain tree leaf extract gel (*Samanea saman*), with an average value of 54.078, while the lowest average was found in the placebo gel control group, with a value of 22.462. Statistical analysis using the Mann-Whitney U test revealed a significant difference between groups ( $p = 0.004$ ,  $p < 0.05$ ). **Conclusion:** A 12% concentration of rain tree leaves (*Samanea saman*) extract gel significantly enhances fibronectin expression, contributing to the healing process of traumatic ulcers.

**KEY WORDS:** Traumatic ulcers, fibronectin, rain tree leaf extract gel (*Samanea saman*), wound healing process.

### **Antiinflamasi gel ekstrak daun trembesi (*Samanea saman*) terhadap ekspresi fibronektin: studi eksperimental**

#### ABSTRAK

**Pendahuluan:** Ulkus traumatikus adalah luka terbuka pada kulit atau mukosa yang sering disebabkan oleh trauma seperti goresan, gigitan, benturan, atau tekanan. Daun trembesi (*Samanea saman*) memiliki potensi mengurangi nyeri dan efek anti-inflamasi berkat kandungan flavonoid. Ekstrak ini bekerja dengan menghambat jalur siklooksigenase dan lipooksigenase, yang membantu membatasi peradangan dan mempercepat proses penyembuhan luka. Tujuan penelitian ini untuk mengetahui peranan gel ekstrak daun trembesi (*Samanea saman*) dengan konsentrasi 12 % terhadap ekspresi fibronektin pada proses penyembuhan ulkus traumatikus. **Metode:** Eksperimental laboratoris dengan desain penelitian post test only control group. Sampel menggunakan hewan coba tikus putih jantan wistar *Rattus norvegicus* dan gel ekstrak daun trembesi yang diekstraksi dengan pelarut etanol 98% menggunakan metode maserasi dengan konsentrasi 12 %. Analisis data menggunakan uji Mann-Whitney U. **Hasil:** Rata-rata tertinggi ditemukan pada kelompok perlakuan yang menerima gel ekstrak daun pohon trembesi 12% (*Samanea saman*), dengan nilai rerata sebesar 54,078, sedangkan rata-rata terendah ditemukan pada kelompok kontrol gel plasebo, dengan nilai sebesar 22,462. Uji Mann-Whitney U menunjukkan bahwa terdapat perbedaan yang signifikan dengan nilai signifikan  $n 0,00 < 0,05$ . **Simpulan:** Pemberian gel ekstrak daun trembesi (*Samanea saman*) 12% berpengaruh terhadap ekspresi fibronektin pada proses penyembuhan ulkus traumatikus.

**KATA KUNCI:** Ulkus Traumatikus, fibronektin, gel ekstrak daun trembesi (*Samanea saman*), proses penyembuhan luka.

## INTRODUCTION

Traumatic ulcers are a common health issue across various age groups, though they occur more frequently in children and physically active individuals.<sup>1</sup> A traumatic ulcer is a lesion or open wound that develops on the skin or mucosa due to various traumatic factors such as scratches, bites, impacts, or pressure.<sup>2</sup> This lesion is a fibrin exudate with a red border and a yellowish center. Ulcers frequently occur due to trauma to the labial mucosa, palate, buccal mucosa, and tongue edge, as well as from thermal injuries, and direct contact with chemicals.<sup>3</sup> Previous research has reported that the prevalence of traumatic ulcers is relatively high compared to other oral lesions. In Indonesia, the prevalence of traumatic ulcers reaches 93.3%.<sup>4</sup>

People often experience prolonged traumatic ulcers or wounds that do not heal properly, resulting in chronic traumatic ulcers. Current treatment for traumatic ulcers generally involves eliminating local factors, such as applying 0.1% triamcinolone acetonide gel as an anti-inflammatory agent that accelerates recovery and manages ulcer pain. However, this treatment can cause side effects such as a burning sensation, stinging, itching, peeling, and oral cavity atrophy. Therefore, alternative treatments with minimal side effects are needed.<sup>5,6,7</sup>

One alternative treatment for traumatic ulcers is phytotherapy, which involves plant-based medicines that provide safe and clinically proven effective care. One plant with potential as an alternative treatment for traumatic ulcers is the rain tree leaf.<sup>6</sup> Phytochemical analysis has revealed that rain tree contains tannins, flavonoids, saponins, steroids, and terpenoids.<sup>8</sup> These active compounds have been identified in rain tree extract, and studies suggest that they possess anti-inflammatory properties and potential to support the healing process. Previous research has demonstrated that rain tree leaf extract (*Samanea saman*) has the potential to reduce pain at a concentration of 12%, due to its active flavonoid compounds.<sup>4,9</sup>

The healing process of traumatic ulcers involves a series of complex biological mechanisms, one of which is the crucial role of an extracellular protein known as fibronectin.<sup>10</sup> Fibronectin is essential for wound healing, performing several key functions that facilitate proper wound healing. It aids in cell adhesion at the injury site, promotes cell migration to the wound area, and supports the formation of the extracellular matrix.<sup>11</sup> Fibronectin contains multiple binding domains, enabling interactions with various molecules and cells within the extracellular environment. These interactions help form bridges that enable cells to interact with the surrounding extracellular matrix. Fibronectin not only helps cells adhere to the injury site but also regulates the interactions of cells with the surrounding extracellular matrix.<sup>12,13</sup>

Therefore, gaining a deeper understanding of fibronectin's role in wound healing could pave the way for the development of more effective therapies and treatment strategies to accelerate the healing process of traumatic ulcers. Given this context, this study aims to evaluate the role of a 12% rain tree leaf extract gel (*Samanea saman*) in the expression of fibronectin during the healing process of traumatic ulcers. This research offers novelty because it utilizes natural ingredients rich in bioactive compounds such as flavonoids and tannins in topical formulations. Its innovation lies in exploring the role of fibronectin, an essential extracellular matrix protein in wound healing process and tissue regeneration, which has received limited attention in previous research. This opens up the potential for the development of scientifically based natural therapies for the management of inflammation and tissue repair. Thus, the primary objective of this research is to analyze the effect of a 12% rain tree leaf (*Samanea saman*) extract gel on fibronectin expression during the healing process of traumatic ulcers.

## METHODS

This study uses a laboratory experimental design with a post-test only control group design. The population of this study consists of male white rats (*Rattus norvegicus*). The sampling technique used is random sampling, calculated using the Lemeshow formula, resulting in a total of 10 rats, divided into two groups.

The Wistar rats (*Rattus norvegicus*), aged 3-4 months and weighing 150-200 grams, were divided into two groups, each consisting of 5 rats per cage. The animals were acclimatized for seven days before treatment administration.

The rain tree leaf extract (*Samanea saman*) was concentrated and then formulated into a gel. The formulation was prepared using CMC-Na as the primary ingredient, followed by the addition of 100 mL of distilled water, and mixed continuously with an electric stirrer for six hours. The concentrated rain tree leaf extract (*Samanea saman*) was then incorporated into the CMC-Na gel to form the 12% rain tree extract gel formulation.

The gel was applied to the labial mucosa of the rats. In Group 1 (control group), the rats received a placebo gel. In Group 2 (treatment group), the rats received the 12% rain tree leaf extract gel. The treatment was applied twice daily, at 9:00 AM and 5:00 PM, topically.

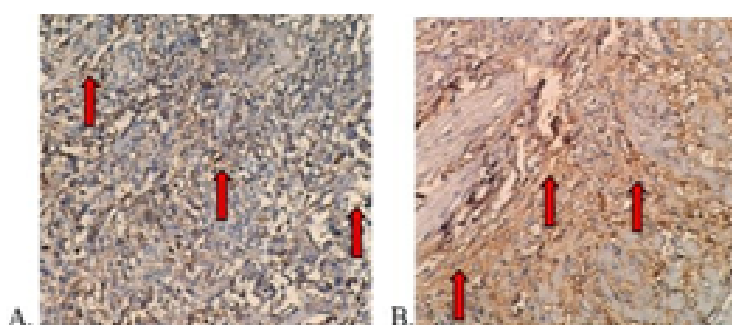
After six hours of treatment, the rats were euthanized by decapitation, and a 3x3 mm biopsy was taken from the labial mucosa of each rat. The samples were placed in a 10% neutral buffered formalin (NBF) fixation tube (1:20 ratio). The samples were stored in a deep freeze (very cold conditions, -80°C) for tissue processing. Paraffin blocks were sectioned at 4 µm, placed in a water bath, and paraffinized. After cutting, the sections were placed intact on glass slides for immunohistochemical examination. Protein expression scoring of fibronectin was assessed visually using a binocular light microscope, using the Kawasaki scoring method<sup>14</sup>, expressed as a semi-quantitative gradation of four scoring results, namely: Score 0: Negative (Presentation 0%). Score 1: Positive 'weak' (Presentation 1-25%). Score 2: Positive 'moderate' (Presentation 26-50%). Score 3: Positive 'Strong' (Presentation 51-75%). Score 4: Positive '-' (Presentation 76-100%). The data obtained from this study were analyzed using the Mann-Whitney U test.

## RESULTS

The highest average was observed in the treatment group receiving the 12% rain tree leaf extract gel (*Samanea saman*), with an average value of 54.078, while the lowest average was found in the placebo control group, with a value of 22.462. The results of this study indicate that the expression of fibronectin in the 12% rain tree leaf extract gel group (*Samanea saman*) was significantly better compared to the placebo control group.

Tabel 1. Mean Fibronectin Expression	
Mean Fibronectin Expression	
Placebo Group	The 12% rain tree leaf extract gel group
22.462	54.078

Fibronectin expression was evaluated microscopically at 400x magnification, and the scoring results for the two groups were as follows:<sup>14,15</sup> Placebo gel control group: Score 1: ('weak') with a percentage 1–25%. Treatment group with 12% Rain tree leaf extract gel (*Samanea saman*): Score 3: ('strong') with a percentage 51–75%. The results of immunohistochemical staining in the placebo control group showed a score of 1 (weak), indicating faint staining intensity and minimal protein expression. In the treatment group with 12% rain tree leaf extract gel (*Samanea saman*), a score of 3 (strong) was observed, indicating intense staining and high, widespread protein expression.<sup>16</sup>



**Picture 1. Fibronectin expression. (A) Placebo group score 1; (B). The 12% rain tree leaf extract gel group score 3.**

The Mann-Whitney U test results showed a significance value of  $0.004 < 0.05$ , indicating a statistically significant difference between the treatment group with 12% rain tree leaf extract gel (*Samanea saman*) and the placebo control group.

**Tabel 2. The Mann-Whitney test results**

	Mean Rank	Sum of Ranks	Significance
Placebo Group	3.00	15.00	0.004
The 12% rain tree leaf extract gel group	8.00	40.00	

## DISCUSSION

The flavonoid compounds in rain tree leaves play a role in wound healing by inhibiting the activity of cyclooxygenase and lipoxygenase, limiting inflammatory cell migration to the wound area and shortening the duration of the inflammatory reaction. Saponins can stimulate the production of type I collagen, which is essential for enhancing epithelialization of tissues and wound closure by preventing excessive tissue formation. Other studies have stated that saponins also contribute significantly to wound healing by stimulating the synthesis of fibronectin by fibroblasts and modulating TGF- $\beta$  receptor expression. The increased expression of fibronectin in response to rain tree leaf extract may be influenced by growth factors such as TGF- $\beta$ , IGF, and VEGF.<sup>16</sup>

The wound healing process is complex and influenced by multiple factors, including time, type, and cell physiology. Physiologically, it occurs in several stages: hemostasis, inflammation, proliferation, and remodeling. During the inflammatory and proliferative phases, fibronectin plays a crucial role in the wound healing process.<sup>17</sup> In the inflammatory phase, fibronectin facilitates blood clot formation at the wound site. When capillaries are damaged, fibronectin aids in the formation of blood clots made of fibrin and fibronectin itself. This blood clot covers the capillary damage and prevents further blood loss.<sup>18</sup> During the proliferative phase, fibronectin serves as a pathway for fibroblasts to migrate to the wound site. Inflammatory cells and platelets release factors such as TGF- $\beta$  and PDGF, which stimulate fibroblasts to migrate to the wound. Fibronectin supports this process by establishing an effective migration pathway.<sup>19</sup>

Fibronectin also plays a key role in extracellular matrix synthesis. Migrating fibroblasts produce protein matrices such as fibronectin, hyaluronan, proteoglycans, and type I and type III procollagen. Fibronectin aids in granulation tissue formation, which fills the wound space. It is also involved in the formation of granulation tissue, a key component of which includes collagen, fibronectin, fibroblasts, endothelial cells, and new blood vessels. Fibronectin contributes to the formation of the structural framework of granulation tissue, which anchors the wound edges.<sup>20</sup>

Previous studies support the finding that rain tree leaf extract has a beneficial effect in accelerating wound healing. A study found that methanol extract from rain tree leaves contains active compounds such as lupeol and epilupeol, which have the potential to be anti-inflammatory and antibacterial, contributing to inflammation reduction and the acceleration of new tissue formation.<sup>21</sup>

Previous research has shown that flavonoid compounds act as potent antioxidants, which may influence pain reduction. The steroid content, which functions as an analgesic, along with the secondary metabolite compounds flavonoids and steroids in rain tree leaf extract (*Samanea saman*), acts as an analgesic by inhibiting the production of inflammatory mediators such as prostaglandins through the suppression of arachidonic acid release, thereby blocking cyclooxygenase enzyme activity. Fibronectin expression can be observed using immunohistochemistry (IHC).<sup>4,22</sup>

Immunohistochemistry (IHC) is a histological staining technique that enables the detection of tissue antigens (markers) in various specimens through the specific interaction between antigen and antibody. Antibodies are used to visualize specific parts of the tissue.<sup>13</sup> The statistical test used was the Mann-Whitney U test. This test is used to determine whether there is a significant difference between the means of two independent samples. It is a non-parametric statistical test that does not require normality

assumptions.<sup>23</sup> The Mann-Whitney U test showed a statistically significant difference between the control and treatment groups. The analysis results indicate that the gel of rain tree leaf extract (*Samanea saman*) influences fibronectin expression.

Further studies are needed to explore its molecular mechanisms, long-term safety, and effectiveness across different wound types. A limitation of this study is the calculation of fibronectin percentage and scoring using a digital application, which requires precision and expertise from the researcher.

## CONCLUSION

The 12% rain tree leaf extract gel (*Samanea saman*) plays a role in increasing fibronectin expression during the healing process of traumatic ulcers. This potential supports innovation in herbal therapy for clinical wound treatment, particularly those requiring stimulation of tissue regeneration, with applications extending to various types of chronic or acute wounds. The implication of this research is that rain tree leaf extract gel could serve as an alternative or complementary treatment for wound care, reducing reliance on synthetic drugs and providing a more biocompatible option with potentially fewer side effects.

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**Data Availability Statement:** Data availability will be granted by the researchers upon request through email correspondence, with consideration for ethical research practices.

**Conflict of Interest:** The authors declare no conflict of interest in this research.

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