

Safety and Efficacy of *Tithonia diversifolia* (Hemsley.) A. Gray Extract Gel in Keloid Patients

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ABSTRACT

Keloids are benign dermal tumors resulting from an exaggerated fibrotic response to skin injury, characterized by dysregulated collagen synthesis and degradation. Traditional treatment options vary in efficacy and are often associated with adverse effects. Preclinical research has identified *Tithonia diversifolia* (Hemsley.) A. Gray as a promising therapeutic candidate due to its ability to inhibit keloid fibroblast proliferation, reduce collagen synthesis, and downregulate TGF β 1 and VEGF expression. Novel treatments that are both effective and safe are critically needed, prompting this investigation into *Tithonia diversifolia* extract. This study aimed to evaluate the safety and efficacy of 2% *T. diversifolia* extract gel as a treatment for keloids, in comparison to the standard 0.025% triamcinolone acetate cream. A 12-week controlled clinical trial was conducted with 14 keloid patients, evenly divided into two treatment groups. Keloid scar characteristics were assessed every 4 weeks using the Vancouver Scar Scale (VSS) and the Patient and Observer Scar Assessment Scale (POSAS). Both the *T. diversifolia* extract gel and triamcinolone acetate cream groups demonstrated significant improvements in total POSAS scores from baseline to week 12. No statistically significant differences were observed between the two treatment groups at the conclusion of the study. Safety evaluations confirmed the tolerability of both treatments over the 12-week period. The 2% *T. diversifolia* extract gel proved to be a safe and effective treatment for keloids, showing comparable efficacy to the 0.025% triamcinolone acetate cream. These findings suggest that *T. diversifolia* extract gel may serve as a viable alternative for keloid management.

Keywords: *Tithonia diversifolia*, keloid, scar, treatment

INTRODUCTION

Keloid, an aggressive and benign fibroproliferative growth of the skin, poses a formidable challenge in clinical practice because of its persistent nature and a high propensity for recurrence following treatment. The management of this skin condition is particularly difficult due to the limited effectiveness of current therapeutic strategies. Although a wide array of interventions is currently available, such as intralesional corticosteroids, cryotherapy, topical agents, and

surgical excision, these methods often fail to provide a permanent cure. Consequently, the rates of keloid recurrence remain a significant concern for both patients and clinicians, with documented rates that vary widely from a considerable 20% to an almost certain 100% (Marneros & Krieg, 2004; Chike-Obi et al., 2009; Davis et al., 2013). This high rate of relapse underscores the urgent need for a more effective and comprehensive understanding of keloid pathogenesis to develop novel therapeutic approaches.

This persistent issue is primarily attributed to the nonspecific nature of current treatment modalities. The underlying pathogenesis of keloid recurrence is complex and involves dysregulated cellular mechanisms such as excessive fibroblast proliferation, altered collagen synthesis, and chronic inflammation (Jumper et al., 2015). These cellular abnormalities, which are not adequately addressed by conventional therapies, lead to the formation of new scar tissue, resulting in high rates of relapse. Consequently, a more comprehensive understanding of these specific cellular processes and the development of targeted therapeutic strategies that can modulate them are critical for reducing the risk of recurrence and significantly improving clinical outcomes in keloid management.

In recent years, there has been growing interest in the use of natural remedies, such as herbal extracts, as potential therapeutic alternatives for keloid treatment. Among these, *Tithonia diversifolia* (Hemsl.) A. Gray, a plant recognized for its anticancer properties, has garnered significant attention. Studies have highlighted the cytotoxic effects of *Tithonia diversifolia* extract and its active component, Tagitinin C, against various cancer cell lines, demonstrating its potential to inhibit abnormal cell proliferation (Gu et al., 2002; Wahyuningsih & Soeparto, 2010; Lee et al., 2011; Liao et al., 2013; Wei et al., 2021; Wahyuningsih & Wijayanti, 2009). Notably, research has also shown that *Tithonia diversifolia* extract inhibits keloid fibroblast proliferation, reduces collagen deposition, and suppresses key growth factors implicated in keloid formation, including Transforming Growth Factor beta (TGF- β) and Vascular Endothelial Growth Factor (VEGF) (Wahyuningsih et al., 2015a; Wicaksana et al., 2020a). Furthermore, preliminary toxicity studies have supported the safety of *Tithonia diversifolia* extract for topical application, further enhancing its potential as a therapeutic agent for keloid treatment (Rizkawati et al., 2022). Despite these promising results, further investigation is needed to validate the efficacy and safety of *Tithonia diversifolia* extract in clinical settings. This study aims to evaluate the therapeutic potential of *Tithonia diversifolia* extract gel in the management of keloids, focusing on its safety and efficacy. By elucidating the benefits of this natural remedy, the study seeks to contribute to the development of alternative treatments for this challenging dermatological condition.

MATERIALS AND METHODS

Study design

This study was conducted at the Department of Pharmacology and Therapy, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, between June 2020 and April 2021. The study design was a randomized, open-label, controlled parallel trial. Randomization was performed using a simple randomization method with a 1:1 allocation ratio (*Tithonia diversifolia* extract gel: triamcinolone acetate cream). Eligible patients meeting the inclusion criteria were enrolled and randomized to one of the treatment groups using a lottery-like system. This ensured equal allocation and minimized potential biases.

Blinding was not feasible due to the distinct color differences between the investigational and control treatments, making it apparent to both patients and examiners which treatment was applied.

Study Participant

The study's inclusion criteria for participants were meticulously defined to ensure a homogeneous and clinically relevant cohort. The study cohort comprised male and female keloid patients within a specific age range of 18 to 60 years. All diagnoses of keloid were professionally confirmed by medical doctors to maintain diagnostic accuracy and consistency. To further refine the study population, a strict set of exclusion criteria was applied. Individuals whose keloids were deemed to require surgical removal were excluded, as were those with hypertrophic scars or scars that originated from burns, which present different pathological characteristics. Furthermore, a number of systemic and patient-specific factors were considered. Pregnant or breastfeeding individuals were not included, nor were patients with any systemic diseases that could potentially interfere with the efficacy or safety of the treatment. A wash-out period was also implemented, excluding patients who had received any form of keloid treatment within the four weeks preceding the study's commencement. Lastly, any keloids that showed signs of active infection or ulceration were also systematically excluded from participation.

Intervention

The investigational product evaluated in the clinical trial was a specialized gel formulated from *Tithonia diversifolia* extract. This formulation, containing a concentration of 2% of the active

extract, is a proprietary compound protected under a formal patent application, specifically identified by the patent number S00202000303, which was filed on January 13, 2020. To serve as a comparative control, the study utilized triamcinolone acetonide cream at a concentration of 0.025%. The treatment protocol was meticulously standardized for both groups. Participants were instructed to first thoroughly cleanse the keloid scar with water, followed by careful drying with a clean cloth or tissue. Subsequently, a thin, uniform layer of either the investigational gel or the comparator cream was to be applied directly to the affected area. This application process was to be performed twice daily, with each application ideally following the participant's regular bathing routine. The total treatment duration was set for a period of three months. To ensure and monitor participant adherence to this regimen, treatment compliance was rigorously assessed during scheduled follow-up visits, which took place at four-week intervals throughout the study. During these visits, the amount of remaining medication in the dispensed tubes was quantitatively measured.

Outcome examination

Outcomes were assessed using the Vancouver Scar Scale (VSS) and the Patient and Observer Scar Assessment Scale (POSAS), following established protocols (Draaijers et al., 2004; Sullivan et al., 1990). Two independent clinicians performed the evaluations within 24 hours, ensuring blinding from each other's results. These assessment tools quantified scar features such as pigmentation, vascularity, and pliability. The combination of patient self-assessments and observer evaluations provided a comprehensive evaluation of scar characteristics. Safety was evaluated through physical examinations and vital sign assessments conducted by medical doctors. Laboratory tests, including liver enzymes (SGOT, SGPT), kidney function (urea, creatinine), and blood glucose levels, were performed by a local ISO-accredited laboratory.

Statistical analysis

The data are presented as mean \pm standard deviation (SD). Statistical analyses were conducted using Prism 9 for macOS software to evaluate the efficacy of *Tithonia diversifolia* extract gel in comparison to 0.025% triamcinolone acetate cream, based on the Vancouver Scar Scale and the Patient and Observer Scar Assessment

Scale. Paired t-test analysis was performed for all paired data, while unpaired t-test analysis was applied to unrelated data. Results with a p-value of < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Baseline characteristic

A total of 14 keloid patients were enrolled and evenly randomized into two groups: 7 participants received the 2% *Tithonia diversifolia* extract gel, and 7 participants received the 0.025% triamcinolone acetate cream. All participants were female, and their vital signs and laboratory test results, including liver enzymes (SGOT, SGPT), kidney function (urea, creatinine), and blood glucose levels, were within normal ranges (Table I). The baseline characteristics were comparable between groups, except for differences in the patients' height.

Clinical efficacy

Vancouver scar scale

The Vancouver Scar Scale was used to measure key parameters of keloid scars, including vascularity, pigmentation, pliability, height, and the overall total score (Figure 1).

The Vancouver Scar Scale was assessed every 4 weeks until week 12. The scale calculations remained consistent between the treated and control groups throughout the study. However, significant improvements in pliability, height, and total score on the Vancouver Scar Scale at week 12 compared to week 0 were observed only in the control group, with p-values of 0.0379, 0.0112, and 0.0084, respectively.

Patient scar assessment scale

The Patient Scar Assessment Scale was administered every 4 weeks until week 12 during the participants' regular visits. Each participant received a printed version of the scar assessment, which they completed during their visit sessions (Figure 2).

The Patient Scar Assessment Scale measurements were consistent between the treated and control groups throughout the study. By the end of the treatment, patients in both groups showed significant improvements in irregularity and total score compared to baseline values. However, significant improvements in pigmentation and thickness were observed only in the control group.

Table I. Baseline characteristic of the study participants. *= $p < 0.05$ vs control (t-test).

No	Parameter	Treated (mean \pm SD) (N=7)	Control (mean \pm SD)(N=7)
1.	Weight (kg)	69.88 \pm 11.57	61.5 \pm 10.09
2.	Height (cm)*	155.63 \pm 4.17	160.88 \pm 3.27
3.	Systolic (mmHg)	123.75 \pm 24.46	116.25 \pm 7.44
4.	Diastolic (mmHg)	78.75 \pm 9.91	76.25 \pm 9.16
5.	Respiratory rate (beat/ min)	18.25 \pm 1.39	17.5 \pm 4.34
6.	Heart rate (beat/minute)	82.13 \pm 18.04	81.5 \pm 9.18
7.	Hemoglobin (g/dL)	14 \pm 1.84	14.75 \pm 1.33
8.	Eritrocytes ($10^6/\mu l$)	4.92 \pm 0.47	5.08 \pm 0.44
9.	Hematocrit (%)	42.38 \pm 4.56	45 \pm 3.25
10.	Leucocytes ($10/\mu l$)	8.9 \pm 2.1	8.9 \pm 2.4
11.	Trombocytes	343750 \pm 64541.79	295750 \pm 51949.84
12.	SGOT	23.38 \pm 11.33	25.25 \pm 11.45
13.	SGPT	27.13 \pm 14.89	32.75 \pm 23.97
14.	Ureum (mg/dL)	22.69 \pm 7.02	20.1 \pm 7.29
15.	Creatinine (mg/dL)	0.73 \pm 0.13	0,79 \pm 0,21
16.	Blood Glucose (mg/dL)	124.75 \pm 85.96	96.25 \pm 37.86

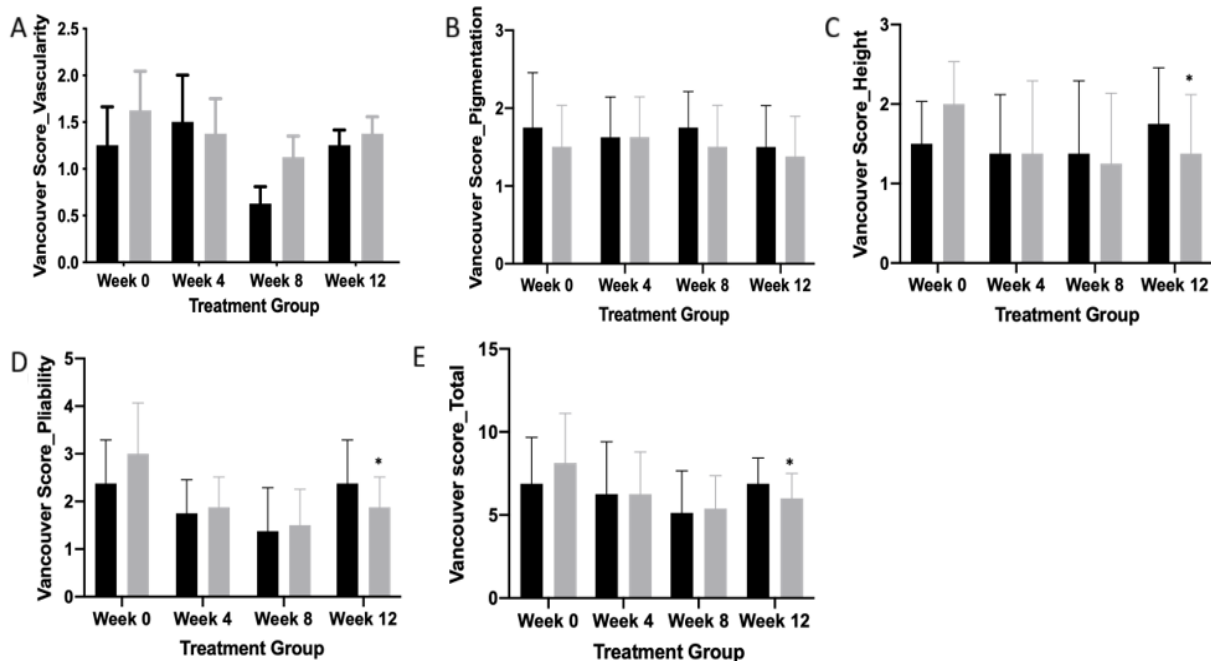


Figure 1. The Vancouver Scar Scale assessment for groups treated with *Tithonia diversifolia* gel (treated group, N=7) and those receiving triamcinolone acetate cream (control group, N=7). The evaluation parameters include Vascularity (A), Pigmentation (B), Height (C), Pliability (D), and Total Score (E) of the keloid scars. An asterisk (*) denotes a significant change ($p < 0.05$) compared to baseline measurements (week 0) as determined by paired t-test analysis. No significant differences were observed between the *Tithonia diversifolia*-treated group and the control group, as analyzed by unpaired t-test.

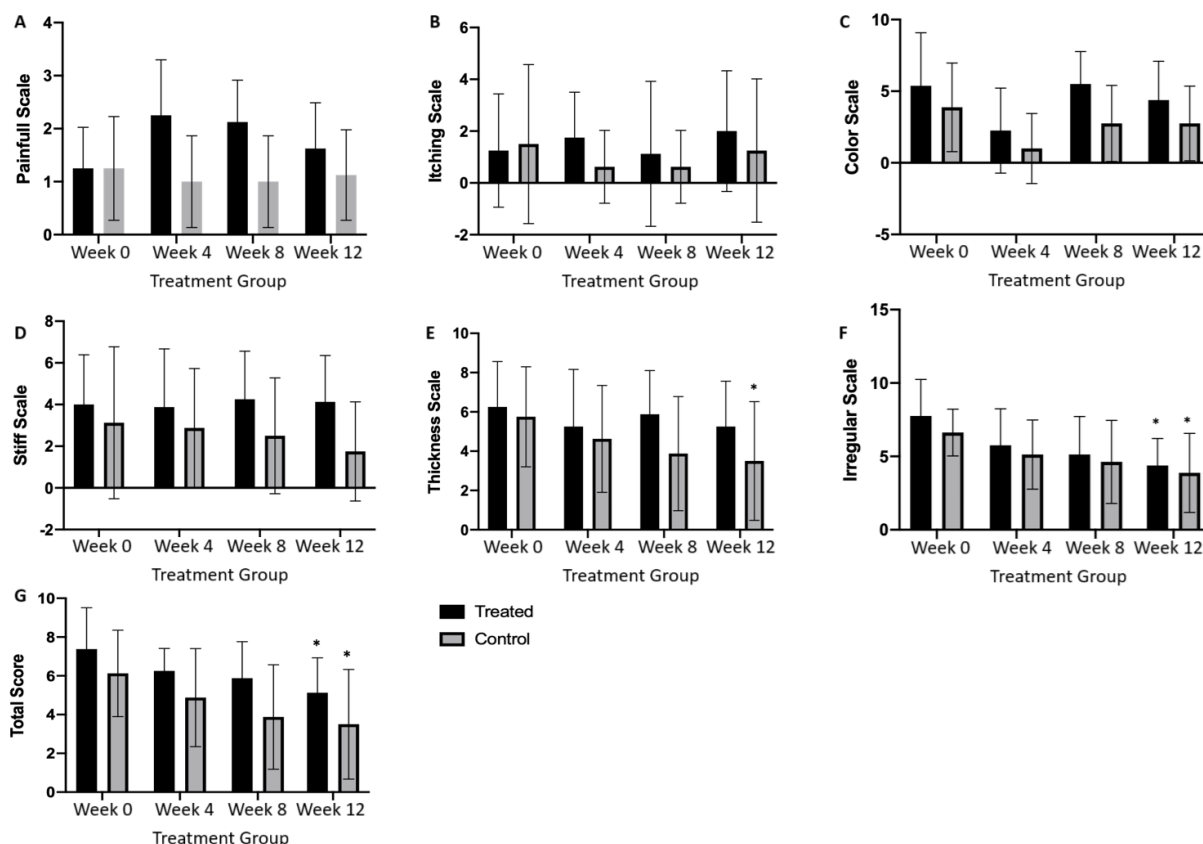


Figure 2. The Patient Scar Assessment Scale for groups treated with *Tithonia diversifolia* gel (treated group, N=7) and those receiving triamcinolone acetate cream (control group, N=7). The evaluation parameters include Pain (A), Itching (B), Color (C), Stiffness (D), Thickness (E), Irregularity (F), and Total Score (G) of the keloid scars. An asterisk (*) denotes a significant change ($p < 0.05$) compared to baseline measurements (week 0), as determined by paired t-test analysis. No significant differences were observed between the *Tithonia diversifolia*-treated group and the control group, as analyzed by unpaired t-test.

Observer scar assessment scale

The Observer Scar Assessment Scale was administered every 4 weeks until week 12 during the patients' regular visits, with evaluations conducted by appointed physicians. The physicians completed printed scar assessment forms during these sessions (Figure 3).

Our study's findings reveal that both the treated and control groups demonstrated significant improvements in several key parameters of the Observer Scar Assessment Scale, specifically in vascularity, relief, pliability, surface, and the overall total score, when compared to their baseline values. However, a notable distinction was found in the evaluation of pigmentation and thickness; significant improvements in these two specific parameters were observed exclusively in the control group. This suggests that while both treatments were effective in addressing certain

aspects of keloid appearance, the control treatment had a more pronounced effect on reducing pigmentation and thickness.

Safety assessment

The safety assessment of the treatments was conducted by monitoring key indicators of the participants' health, including vital signs and blood parameters. The results showed no discernible differences between the treatment and control groups across all monitored metrics. Specifically, there were no significant changes observed in systolic and diastolic blood pressure, respiratory rate, heart rate, or the results from the comprehensive laboratory examinations, as detailed in Table II. These findings suggest that both the investigational gel and the comparator cream were well-tolerated by the study participants.

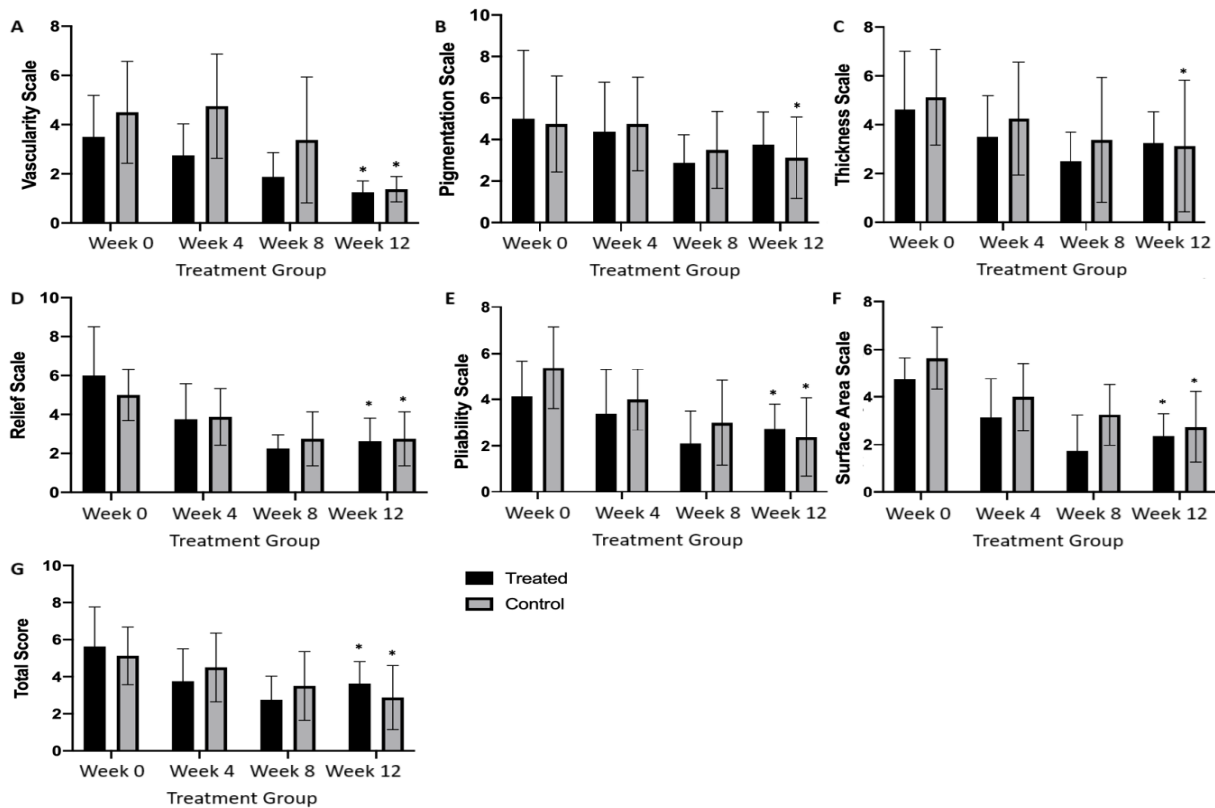


Figure 3. The Observer Scar Assessment for groups treated with *Tithonia diversifolia* (treated, N=7) and those receiving triamcinolone acetate (control, N=7). The evaluation parameters displayed in each image include Painfull (A), Itching (B), Color (C), Stiff (D), Thickness (E), Irregularity (F), and the Total Score (G) for patient's keloid. An asterisk (*) indicates a significant change with $p < 0.05$ compared to the baseline (week 0, paired T test). No significant differences were observed between the *Tithonia diversifolia* -treated (treated) group and the control group (non-paired T test).

Table II. The safety parameters of the groups measured at the end of the study.

No.	Parameter	Treated (mean ±SD)	Control (mean ± SD)
1.	Systolic Blood Pressure (mmHg)	108.75±16.67	108.75±12.13
2.	Diastolic Blood Pressure (mmHg)	71.88±7.99	72.5±10.35
3.	Respiratory rate (beat/ minute)	17±1.41	16.5±2
4.	Heart rate (beat/minute)	78.75±9.45	80.5±6.99
5.	Hemoglobin (g/dL)	13.89±1.38	14.68±1.91
6.	Eritrocytes ($10^6/\mu l$)	4.85±0.38	4.94±0.63
7.	Hematocrit (%)	42.12±3.64	4.94±0.63
8.	Leucocytes	10276.25±2217.64	8847.5±2342.59
9.	Trombocytes	341125±71272.99	296000±58726.97
10.	SGOT	22.25±6.36	23±9.94
11.	SGPT	28.5±14.78	31.75±27.95
12.	Ureum (mg/dL)	23.85±5.40	18.31±6.78
13.	Creatinine (mg/dL)	0.75±0.11	0.80±0.19
14.	Blood Glucose (mg/dL)	114±93.33	98.63±46.96

The only complaint reported in the treated group was discoloration of the area where the gel was applied, attributed to the herb-derived color of the *Tithonia diversifolia* gel

Our study utilized two complementary methodologies to evaluate keloids: the Vancouver Scar Scale (VSS) and the Patient and Observer Scar Assessment Scale (POSAS). The VSS, initially developed for assessing burn scars, measures key clinical parameters such as vascularity, thickness, pliability, and pigmentation from a clinician's objective perspective. In contrast, the POSAS expands the scope of evaluation by incorporating a more holistic set of factors. It includes both objective and subjective assessments of the scar, covering parameters like surface area, pain, itching, color, stiffness, and thickness. Most importantly, the POSAS incorporates the patient's subjective perception of their relief and overall satisfaction, which is crucial for a comprehensive understanding of the treatment's impact (Park et al., 2022; Lyons et al., 2023). This dual approach allows for a robust and thorough evaluation of keloid outcomes from both a clinical and patient-centered viewpoint.

Over a 12-week period, the effectiveness of *Tithonia diversifolia* extract gel and 0.025% triamcinolone acetate cream in treating keloids was evaluated. Based on the Vancouver Scar Scale and the Patient and Observer Scar Assessment Scale (POSAS), no significant differences were observed between the two treatments overall. However, the group treated with 0.025% triamcinolone acetate cream exhibited significant improvements in scar pliability and height according to the Vancouver Scar Scale. Using POSAS, both treatments demonstrated enhancements in certain scar parameters throughout the study. These findings suggest that the 2% *Tithonia diversifolia* extract gel is comparably effective to the 0.025% triamcinolone acetate cream in modifying specific scar characteristics. Nevertheless, triamcinolone acetate cream outperformed *Tithonia diversifolia* gel in improving keloid pigmentation and reducing scar thickness. This discrepancy may be attributed to the pharmacodynamic properties of *Tithonia diversifolia* gel, which appears more effective during the early stages of keloid formation when Transforming Growth Factor Beta 1 (TGF- β 1) and Vascular Endothelial Growth Factor (VEGF) activity are heightened (Wahyuningsih et al., 2015b; Astuti & Wahyuningsih, 2019; Wicaksana et al., 2020b; Santi et al., 2019). In contrast, the keloids assessed in this study were mature, with lower TGF- β 1 and VEGF activity compared to early-stage keloids (McGinty & Siddiqui, 2023). Triamcinolone acetate, like other corticosteroids, is well-known

for its vasoconstrictive and depigmenting properties, which contribute to its efficacy in improving keloid pigmentation and thickness (Juckett & Hartman-Adams, 2009; Roquest & Teot, 2008). These characteristics may explain the superior performance of triamcinolone acetate cream in these aspects compared to *Tithonia diversifolia* gel.

Keloids arise from an abnormal wound healing process characterized by prolonged inflammatory and fibroblastic phases, followed by a delayed maturation phase (McGinty & Siddiqui, 2023). This process is marked by an imbalance between collagen synthesis and extracellular matrix degradation, driven largely by elevated levels of inflammatory mediators such as Transforming Growth Factor Beta 1 (TGF- β 1) and its receptor, as well as Vascular Endothelial Growth Factor (VEGF). These factors play a pivotal role in promoting aberrant fibroblast activity and excessive angiogenesis, which are hallmarks of keloid pathology, particularly during the early stages of keloid formation (Berman et al., 2017; Betarbet & Blalock, 2020; Wu et al., 2004; Wilgus et al., 2008).

Safety evaluations indicated no significant differences in vital signs or laboratory test results between participants treated with *Tithonia diversifolia* gel and those in the control group. Additionally, a sub-chronic in vivo toxicity study supported the safety of prolonged dermal application of *Tithonia diversifolia* extract (Rizkawati et al., 2022). The only reported adverse effect was temporary discoloration of the skin at the application site, which could be easily removed with soap and water.

The study provides novel insights into the therapeutic potential of *Tithonia diversifolia* for keloid treatment, representing a significant strength. However, certain limitations must be acknowledged. The assessment was confined to mature keloids, leaving its effects on early-stage or active keloids unexplored. Future research should focus on understanding the mechanisms through the findings from this study suggest that the investigational gel, formulated with *Tithonia diversifolia* (Hemsley) A. Gray, shows promise as a potential alternative treatment for keloids. This is particularly relevant for addressing early keloid development and active wound sites, where the biological activity of key growth factors like TGF- β 1 and VEGF is significantly heightened. The therapeutic effects observed, especially in a clinical setting, pave the way for a new natural approach to

a persistent and challenging skin condition. However, to more definitively establish its optimal therapeutic applications, dosage, and long-term efficacy, it is imperative that larger, randomized controlled trials are conducted. Furthermore, these future studies should incorporate extended follow-up periods to thoroughly assess the sustained benefits and the potential for long-term recurrence prevention in patients. This ongoing research is critical for enhancing the quality of care and improving clinical outcomes for those who suffer from keloids.

CONCLUSION

This study identifies *Tithonia diversifolia* (Hemsley) A. Gray as a highly promising natural treatment candidate for keloids, providing a potential and much-needed alternative to existing therapeutic options. These significant findings lay the groundwork for a new direction in research, with the ultimate goal of developing more effective and targeted interventions. By exploring the therapeutic properties of this plant, this research aims to enhance the quality of care and improve long-term outcomes for individuals struggling with this challenging and persistent skin condition.

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CONFLICT OF INTEREST

The treatment preparation was provided by the Pharmaceutical Technology Laboratory, which was not involved in the design, conduct, interpretation, or publication of the study. The authors declare no competing interests.

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