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Formulation and Potency Extract Gel of Citrus Mistletoe Leaves (*Dendrophthoe glabrescens* (Blakely) Barlow) on Burn Wound Healing

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ABSTRACT

Second-degree burns require effective topical therapy to accelerate tissue regeneration and prevent infection. Mistletoe leaves (*Dendrophthoe glabrescens* (Blakely) Barlow) are traditionally used and are known to be rich in secondary metabolites such as flavonoids, saponins, and tannins, which have anti-inflammatory and antioxidant potential. This study aimed to evaluate the effectiveness of an ethanol leaf extract gel of (*Dendrophthoe glabrescens* (Blakely) Barlow) in healing second-degree burns in white mice (Mus musculus). The gel was formulated at extract concentrations of 5%, 10%, and 15%. A posttest-only control group design was employed, and the extract gel was applied for 14 days. The results demonstrated a significant increase in the percentage of wound healing, particularly at the 15% concentration, which approached the efficacy of the positive control. The flavonoid, saponin, and tannin activities in the (*Dendrophthoe glabrescens* (Blakely) Barlow) leaf extract play crucial roles in the healing process through anti-inflammatory, antibacterial, and antioxidant mechanisms, as well as by enhancing collagen synthesis. These findings suggest that this extract gel has potential as an effective alternative therapy for burn wounds.

***Keywords*:** Citrus mistletoe leaves; Gel; Burn wounds; Wound healing

INTRODUCTION

A burn is an injury that can damage the skin and disrupt the body's homeostasis.[1] The majority of burn injuries are caused by heat from hot liquids, solids, or fire, but they can also be caused by friction, cold, heat, radiation, chemicals, or electricity. According to WHO data from 2018, there are about 265,000 burn-related deaths every year. Over 90% of these deaths occur in low- and middle-income African and Asian nations. According to Riskesdas data from the Republic of Indonesia's Ministry of Health, the prevalence of wounds in Indonesia increased from 8.2% in 2013 to 9.2% in 2018. Because wounds are so common in Indonesia, medications for wound healing are necessary.[2] Restoration of the skin's anatomical and functional integrity after a burn requires proper wound healing.[3] During wound healing, the body undergoes a complex physiological process in which the skin or organ repairs itself after injury.[4] This process consists of four overlapping phases: hemostasis, inflammation, proliferation, and remodeling. Hemostasis, or the pro-inflammatory phase, is the first and shortest phase, during which bleeding is

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stopped.[5] Once hemostasis is achieved, vasodilation and capillary leakage occur, releasing local histamine via the activated complement cascade, leading to migration of inflammatory cells to the wound site and inflammation.[6] After inflammation subsides, the body releases various cells responsible for migration and proliferation. Finally, the maturation or remodeling phase may last months to years, during which fibroblasts leave the wound bed and collagen is reorganized into a more structured matrix. Consequently, wound healing is a lengthy process and is highly susceptible to microbial infection.[7] A complex network of interacting pro-inflammatory cytokines, chemokines, growth factors, and receptors/ligands on different cells controls these four phases. The length of time it takes for a wound to heal varies depending on the individual and the severity of the injury.[8]

A commonly used treatment for burns is placenta extract 10%, neomycin sulfate 0,5%, and a gel base. This medication may cause skin irritation, marked by red spots, and typically requires large quantities for application to the wound site. The high prevalence of burns and their complications, as well as the financial burden on families and healthcare systems, underscore the need for more affordable and effective alternative therapies.[9]

As a result, the investigation of more economical, secure, and efficient alternative treatments is required due to the high frequency of burns and their sequelae. By utilizing centuries' worth of traditional knowledge, herbal medicine presents a potential and economical solution.[10]

Citrus mistletoe leaves are a parasitic plant that derives nutrients and defensive compounds from its host, causing its secondary metabolite profile to adapt to the host plant.[11] Ethanol extracts of citrus mistletoe leaves contain secondary metabolites such as alkaloids, steroids, flavonoids, saponins, and tannins.[12] During wound healing, flavonoids act directly as broad-spectrum antibiotics and antioxidants that combat free-radical damage, exhibit anti-inflammatory effects by reducing IL-1 and TNF-α levels and activating macrophages.[13] Saponins stimulate collagen formation by influencing fibroblasts. Alkaloids provide antibacterial action by intercalating cell walls or DNA and promote fibroblast precursor formation, thus enhancing collagen synthesis. Tannins function as antioxidants, antimicrobials, angiogenic agents via upregulation of VEGF-A (*Vascular Endothelial Growth Factor-A)* gene expression, and as astringents that constrict skin pores and reduce exudate and mild bleeding.[14]

Conventional formulations commonly used for wound treatment include gel preparations.[15] Gel formulations offer numerous advantages, such as precise dosage; an appealing form and appearance; elastic consistency, and efficient drug release. Gels have a high water content, making them non-sticky, hydrating the epidermal layer to prevent skin irritation, and enhancing the penetration of active ingredients.[16] Based on the above explanation, this study was conducted to determine the effectiveness and the most effective concentration of ethanol extract gel from *Dendrophthoe glabrescens (Blakely)* Barlow in the healing of second-degree (IIA) burn wounds in white mice (Mus musculus). Additionally, this study aims to evaluate the physical quality of the ethanol extract gel from *Dendrophthoe glabrescens (Blakely)* Barlow.

METHODS

Equipment

Beaker glass (Pyrex), stirring rod (Pyrex), oven, aluminum foil, rotary evaporator (IKA RV 10), porcelain dish (Pyrex), mortar and pestle, induction stove, water bath, analytical balance (Ohaus), digital scale, measuring glass (Pyrex), caliper, hair shaver (Gillette), round metal plate, flannel cloth, climatic chamber Memmert HPP 110Eco, glass plate, extensometer, gel pot.

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Materials

Citrus mistletoe leaves (*Dendrophthoe glabrescens* (Blakely) Barlow) obtained from Manikliyu Village, Kintamani, Bali. Ethanol 96% (PT. Brataco, Indonesia) as the solvent and other gel-making ingredients such as Carbopol (PT. Brataco, Indonesia), triethanolamine, propylene glycol (PT. Brataco, Indonesia), glycerin (PT. Brataco, Indonesia), benzoic acid (PT. Brataco, Indonesia), and distilled water (UD. Sekawan Bali Sejahtera, Indonesia). Other materials used include ketamine and xylazine as anesthetics, filter paper (Whatman), parchment paper, pH meter paper or pH meter (Ohaus), Mayer's reagent, Dragendorff reagent, Liebermann-Burchard reagent, concentrated HCl, 1% $FeCl_3$, 70% alcohol, *Bioplasenton*® gel, white mice, and cotton/tissue for cleaning equipment.

Research Procedure

This study is a true experimental study with a posttest-only control group design. The research process consists of several steps, as explained below.

Plant Preparation and Identification

The sample used was 5 kilograms of fresh citrus mistletoe leaves (*Dendrophthoe glabrescens* (Blakely) Barlow) collected from Manikliyu Village, Kintamani, Bali. The sample was identified at the Biological Research Center, National Research and Innovation Agency (BRIN), Eka Karya Botanical Garden, Bedugul with transaction number: 1617-80983-1, February 27, 2023.

Extraction Process

A total of 5 kg of fresh leaves was weighed and washed twice with running water. The clean leaves were chopped, drained, and weighed to determine their wet weight. The leaves were then dried in an oven at 45°C for 6 hours until completely dry. After drying, the leaves were ground using a blender to produce a powdered simplicia. A total of 650 grams of powdered simplicia was soaked in 96% ethanol solvent at a 1:10 ratio (material to solvent) for 3 days using the maceration method. The maceration result was then filtered using flannel cloth or filter paper and concentrated using a vacuum rotary evaporator to obtain a thick extract. This remaceration process was repeated twice. The final extract was weighed and stored in a sealed container before use in further testing. [17]

Yield Calculation

The extract yield calculation method is used to determine the percentage of citrus mistletoe leaf extract obtained per gram of dried powder. The following formula can be used to calculate the yield percentage: [18]

$$\%\ Rendemen = \frac{Weight\ of\ extract\ obtained}{Initial\ weight}\ x\ 100\%$$

The phytochemical screening conducted includes tests for alkaloids, triterpenoids/steroids, flavonoids, saponins, and tannins.

Alkaloids

The extract is placed in three test tubes—one as a control and the other two tested with Mayer's reagent and Dragendorff's reagent. The presence of a white or yellowish-white precipitate after adding Mayer's reagent and an orange-red precipitate after adding Dragendorff's reagent indicates the presence of alkaloids. [19]

Steroids/Triterpenoids

The extract is divided into two test tubes—one as a control and the other mixed with Liebermann-Burchard reagent. A red or purple color change indicates the presence of triterpenoids, while a green or blue color change indicates the presence of steroids.[20]

Flavonoids

The extract is placed in two test tubes—one as a control and the other mixed with 5–6 drops of concentrated HCl. The appearance of a red or yellow color indicates the presence of flavonoids.[21]

Saponins

The extract is placed in two test tubes—one as a control and the other boiled with 20 mL of water in a water bath. After boiling, the filtrate is shaken and left to

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stand for 15 minutes. The formation of foam indicates the presence of saponins.[15]

Tannins

The extract is placed in a test tube and mixed with 2–3 drops of 1% $FeCl_3$ solution. A blackish-green color change indicates the presence of tannins.[22]

Preparation of Ethanol Gel from Citrus Mistletoe Leaf Extract

The gel formulation consists of three different formulas, each varying in extract concentration as the active ingredient at 5%, 10%, and 15%.[23] The formulation details are presented in the table below.

Gel Preparation of Ethanol Extract from Citrus Mistletoe Leaves

The first step is to prepare the necessary equipment and materials, then weigh the ingredients as required. The gel preparation begins by dissolving carbopol in a portion of distilled water in a mortar, then triturating until it forms a gel base (*mucilage*). Triethanolamine, glycerin, and DMDM hydantoin are then gradually added to the mortar and stirred until homogeneous. Next, propylene glycol mixed with citrus mistletoe leaf extract is added to the mortar and stirred until a uniform gel mass is obtained. Finally, distilled water is added until the gel mass reaches 100 g, and the formulation is then packaged in a container. [24]

Physical Quality Testing of Ethanol Extract Gel from Citrus Mistletoe Leaves

1. Stability Test (Cycling Test)

The stability test was conducted using the temperature cycling test method over six cycles. Each cycle involved storing the gel in a climatic chamber at 40±2°C for 24 hours, followed by 4±2°C for 24 hours. Physical stability was assessed based on organoleptic properties, viscosity, pH, homogeneity, spray pattern, spreadability, and adhesiveness. These parameters were observed before and after the sixth cycle of the temperature cycling test.[25]

2. Organoleptic Test

The organoleptic test was conducted before and after the cycling test by observing color, aroma, and consistency changes.[26]

3. pH Measurement

The gel's pH was measured using universal pH indicator paper. The observed pH should match human skin pH (4.5–6.5).[27]

4. Homogeneity Test

The homogeneity test was performed by spreading the gel on a glass slide to observe particle distribution. The gel is considered homogeneous if it is well-mixed without visible coarse particles. [28]

Table 1. Formulation of Ethanol Gel Preparation from Citrus Mistletoe Leaf Extract

Component	Function	Concentration (% w/w)		
		F1 (5%)	F2 (10%)	F3 (15%)
Ethanol extract of orange benalu leaves	Active substances	5	10	15
Carbopol	Gelling agent	2	2	2
Propilen glikol	Cosolvent	5	5	5
Gliserin	Humectan	10	10	10
DMDM Hydantoim (ml)	Preservatives	0.6	0.6	0.6
Trietanolamin	Emulsifier	q.s	q.s	q.s
Aquadest	Solvent	Ad 100	Ad 100	Ad 100

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5. Spreadability Test

The spreadability test was conducted by placing 0.5 grams of gel in the center of an extensometer, covering it with a glass plate, and leaving it for 1 minute before measuring the initial diameter. A 150-gram weight was then applied for 1 minute, and the final diameter was recorded. A good gel formulation should have a spreadability diameter between 5–7 cm.[29]

6. Adhesiveness Test

The adhesiveness test was performed by placing 0.25 grams of gel on a glass slide of known area, covering it with another glass slide, and applying a 1000-gram load for 5 minutes. Then, a 50-gram counterweight was used to pull the slides apart, and the time until separation was recorded. The optimal adhesiveness for topical preparations is more than 4 seconds. [30]

Induction of Second-Degree Burns (IIA) in Mice

Before treatment, the mice were acclimatized for 7 days. The procedure began with administering anesthesia using an intramuscular injection of ketamine (40 mg/kg BW) and xylazine (5 mg/kg BW).[31] The fur on the mice's back was then shaved over an area of approximately 3–5 cm, and an aseptic procedure was performed using 70% alcohol. To create second-degree burns (IIA), a 2 cm diameter, 1 mm thick metal rod was heated in boiling water at 100°C for 3 minutes and then applied to the mice's back for 10 seconds.[32]

Application of Citrus Mistletoe Leaf Extract Gel

The burns on the mice's back were treated with different therapies according to the group classifications: negative control (K-): Treated with the gel base (without extract), positive control (K+): Treated with *Bioplasenton*® (Placenta extract 10% and Neomycin sulfate 0.5%.) gel, treatment group 1 (P1): Treated with 5% ethanol extract gel of citrus mistletoe leaves, treatment group 2 (P2): Treated with 10% ethanol extract gel of citrus mistletoe leaves, treatment Group 3 (P3): Treated with 15% ethanol extract gel of citrus mistletoe leaves. The topical therapy was applied in a dose of 0.3 grams, evenly distributed over the burn wound once daily at 18:00 WITA for 14 days.[33]

Measurement of Burn Diameter

The measurement of the burn area was conducted using a caliper. The burn wound diameter on day x was calculated using the following formula:

$$dx = \frac{d1 + d2 + d3 + d4}{4}$$

Note:
dx : X-day wound diameter
d1 : Wound diameter 1
d2 : Wound diameter 2
d3 : Wound diameter 3
d4 : Wound diameter 4

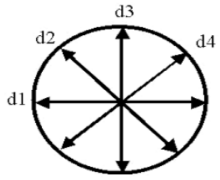

Figure 1. Method for Measuring Burn Wound Diameter

Observation and Measurement

The percentage of burn wound healing is determined based on the reduction in wound diameter in the tested mice. Observation and measurement of the burn wound area were conducted by measuring the wound diameter (mm) in each mouse, once daily for 14 days. The obtained data were then used to calculate the percentage of burn wound healing using the following formula:

Percentage of Burn Wound Healing

$$\frac{L1 - Ln}{L1}\ x\ 100$$

Note:
L1 : Extent of the wound on the first day
Ln : Extent of the wound on the n day.

Data Analysis

The research data were analyzed using SPSS version 27. The analysis began with the Shapiro-Wilk test to examine the normality of the data distribution, followed by a Levene's test for homogeneity. To determine whether there were significant

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differences between two or more groups, a One-Way ANOVA test was performed, followed by a post hoc LSD test.[34]

RESULTS AND DISCUSSION

This study has been ethically approved by the Research Ethics Committee of Politeknik Kesehatan Denpasar, as stated in the Ethical Clearance Certificate No. DP.04.02/F.XXXII.25/0920/2024. The Characterization Laboratory of Eka Karya Botanical Garden, Bali-BRIN, conducted the determination and identification of the plant species used in this study, *Dendrophthoe glabrescens* (Blakely) Barlow. This plant has scientific synonyms such as *Loranthus longiflorus var. savannorus Domin*, *Loranthus vitellinus var. glabrescens Blakely*, and *Dendrophthoe pelagica Barlow*.

A total of 650 grams of citrus mistletoe leaves were macerated using 96% ethanol until the solution was nearly colorless. The resulting filtrate was then concentrated using a rotary evaporator, yielding 125.71 grams of thick extract with a yield of 19.34%. This yield value represents the ratio of the extract obtained to the extracted sample, where a higher yield percentage indicates a higher content of bioactive compounds in the extract. This yield meets the minimum requirement of 10% for thick extracts.[35] Once an appropriate thick extract was obtained, phytochemical screening was performed. The screening was conducted to identify secondary metabolites present in the ethanol extract of citrus mistletoe leaves. The results were consistent with previous research by[36], which stated that the ethanol extract of citrus mistletoe leaves contains alkaloids, steroids, flavonoids, tannins, saponins, and steroids.[37]

The organoleptic test was conducted to assess the form/consistency, color, and aroma of the gel containing citrus mistletoe leaf extract. The results showed that all gel formulations had a semi-solid consistency, which is characteristic of gels in general.

Table 2. Phytochemical Screening Results Extract of *Dendrophthoe glabrescens* (Blakely) Barlow

Phytochemical	Reagents	Extract Contect
Alkaloids	Mayer and Dragendorff	(+)
Flavonoids	Magnesium sulfate and Hydrochloric acid	(+)
Tanins	Ferric chlodride	(+)
Saponins	Distilled water	(+)
Triterpenoids	Acetic acid anhydride+Sulfuric acid	(-)
Steroids	Acetic acid anhydride+Sulfuric acid	(+)

Note : (+) Present and (-)Absent

The gel appeared green due to the ethanol extract of citrus mistletoe leaves. However, the resulting gel was not clear or transparent due to the dense color of the extract. The gel color varied according to extract concentration: light green at 5%, dark green at 10%, and blackish-green at 15%. Therefore, it can be stated that the higher the extract concentration, the darker the gel color. Additionally, increasing the extract concentration enhanced the characteristic aroma of citrus mistletoe leaves.[38] After undergoing a cycling test, no changes were observed in the gel's color, form/consistency, or aroma, indicating that the citrus mistletoe leaf extract gel has good stability during storage.[39] The pH test was conducted to ensure that the topical preparation had an appropriate pH range, specifically between 4.5 and 6.5. The test results showed that all three concentrations of ethanol extract gel, both before and after the cycling test, met the required pH standard of 5.

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Table 3. Physical Quality Test Extract Gel

Test Parameters	Before Cycling Test			After Cycling Test		
	F1 (5%)	F2 (10%)	F3 (15%)	F1 (5%)	F2 (10%)	F3 (15%)
Organoleptic						
Colour	Light green	Dark green	Blackish green	Light green	Dark green	Blackish green
Aroma	Specific citrus mistletoe leaves	Specific citrus mistletoe leaves	Specific citrus mistletoe leaves	Specific citrus mistletoe leaves	Specific mistletoe leaves	Specific citrus mistletoe leaves
Consistency	Semi solid	Semi solid	Semi solid	Semi solid	Semi solid	Semi solid
pH	5	5	5	5	5	5
Homogeneity	homogeneous	homogeneous	homogeneous	homogeneous	homogeneous	homogeneous
Spreadability	4.2	4.2	4.2	3.9	4	4.15
	4.6	4.65	4.7	4.2	4.4	4.65
Adhesive	6.29	10.48	12.17	5.65	9.01	11.68

Figure 2. *Dendrophthoe glabrescens* (Blakely) Barlow leaf extract gel

A topical formulation with a pH that is too low can cause skin irritation, whereas an overly alkaline pH can lead to skin dryness and flaking.[40] Additionally, pH influences drug release; if the formulation's pH does not match the skin's pH, the active ingredient may ionize, making it difficult to penetrate the skin.[41] Therefore, the gel formulation should have a pH similar to that of the skin to ensure the active ingredient remains non-ionized and easily absorbed.[42]

The homogeneity test was performed to ensure that the formulation was uniform, as a well-formulated gel should be free of clumps and coarse particles.[43] The results before and after the cycling test showed that all three gel concentrations were homogeneous, as no coarse particles were detected under the microscope. A homogeneous gel ensures even distribution of the active ingredient, which is expected to provide consistent effectiveness upon application.[44]

The spreadability test was conducted to evaluate how easily the gel spreads when applied to the skin. Before the cycling test, F1 had a spreadability of 4.6 cm, F2 was 4.65 cm, and F3 was 4.7 cm (Table 3). After the cycling test, F1 had a spreadability of 4 cm, F2 was 4.4 cm, and F3 was 4.65 cm (Table 3). This result is close to the standard value, as an ideal gel should have a spreadability between 5-7 cm. This limitation was attributed to Carbopol, a polymer with a high molecular weight that increases viscosity, thereby reducing gel spreadability.[45] A high glycerin concentration can also increase gel viscosity by binding more water and enlarging molecular size, leading to higher resistance to flow and spreading.

The adhesiveness test results before the cycling test showed that F1 had an adhesion time of 6.29 seconds, F2 had 10.48 seconds, and F3 had 12.17 seconds. After the cycling test, F1 had 5.65 seconds, F2 had 9.01 seconds, and F3 had 11.68 seconds (Table 3). These results met the required standard, as an ideal topical formulation should have an adhesion time of more than 4 seconds. Gel adhesiveness is related to viscosity, where higher viscosity allows the gel to remain on the skin surface longer,

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thereby influencing drug diffusion from the gel base to the skin surface.[46]

Burn Wound Healing

The percentage difference in burn wound diameter between day 14 and day 1 for each group is presented in Table 4. The graph in Figure 2 shows that the positive control group (K+) exhibited the highest percentage reduction in wound diameter (79%), followed by the treatment group 3 (P3) at 77%, treatment group 2 (P2) at 76%, treatment group 1 (P1) at 75%, and the negative control (K-) at 56%.

Based on the normality test results using the Shapiro-Wilk method, the wound diameter difference data in all groups were normally distributed, with significance values (p) for K(-), K(+), P1, P2, and P3 of 0.757, 0.959, 0.420, 0.744, and 0.220, respectively ($p > 0.05$). The homogeneity test results using Levene's test also indicated homogeneous variance, with a significance value of 0.148. Furthermore, the One-Way ANOVA test yielded a significance value of < 0.001 ($p < 0.05$), indicating that at least two groups showed significant differences in the percentage of burn wound diameter reduction. In the follow-up analysis using Post Hoc Tukey, a significant difference was found between K(-) and K(+), P1, P2, and P3 ($p < 0.001$). However, the post hoc results showed that K(+) did not significantly differ from P1, P2, and P3, with significance values of 0.053, 0.120, and 0.295, respectively. This indicates that the effects of P1, P2, and P3 were comparable to the positive control (K+), suggesting the potential effectiveness of active compounds in burn wound healing.

Based on the data above, it can be concluded that the negative control group exhibited the slowest burn wound healing process compared to other groups. This is because the negative control group lacked active compounds that aid in wound healing and allowed for a prolonged risk of microbial infection, which delayed the healing process. In contrast, the positive control group showed faster wound healing due to the presence of 10% placenta extract and 0.5% neomycin sulfate in bioplacenton gel. Placenta extract acts as a biogenic stimulator that accelerates cell regeneration, wound healing, reduces transforming growth factor, and increases vascular endothelial growth factor and CD31+ expression. Meanwhile, neomycin sulfate serves as an effective antibiotic.[47]

Furthermore, the treatment using ethanolic extract gel from citrus mistletoe leaves demonstrated that higher extract concentrations in the formulation resulted in a wound healing efficacy closer to that of the positive control. This is because higher amounts of bioactive compounds contribute to faster wound closure and enhanced antimicrobial strength, which further accelerates the healing process.[48]

Table 4. Percentage Difference in Wound Diameter (%)

K (-)	K (+)	P1	P2	P3
59.7	78.2	76.93	76.07	78.71
58.1	80.09	70.65	73.71	78.3
51.22	77.4	75.18	74.92	76.95
54.43	78.91	75.98	77.67	73.07
55.86±3.80	**78.65±1.14**	**74.98±2.20**	**75.77±1.79**	**76.75±2.57**

Note: K(-): Negative Control Group (Treated with the gel base (without extract), K(+): Positive Control Group (Treated with *Bioplasenton*® gel), P1 (Treatment Group 1) : Treated with 5% ethanol extract gel of citrus mistletoe leaves, P2 (Treatment Group 2) : Treated with 10% ethanol extract gel of citrus mistletoe leaves, P3 (Treatment Group 3) : Treated with 15% ethanol extract gel of citrus mistletoe leaves.

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Burn wounds damage the integrity of the skin, triggering various reactions such as pain, inflammation, oxidative stress, infections, and other adverse effects that can directly or indirectly slow down healing. Therefore, topical treatments for burns should possess anti-inflammatory, antioxidant, antibacterial properties, and the ability to enhance collagen production. One plant with such potential is citrus mistletoe, as its ethanolic leaf extract contains secondary metabolites such as alkaloids, steroids, flavonoids, saponins, and tannins, all of which support wound healing.[49]

Inflammatory Phase and the Role of Flavonoids, Saponins, and Tannins

The inflammatory phase is a crucial initial stage in the wound healing process. During this phase, neutrophils and monocytes migrate to the inflammation site, triggered by inflammatory mediators.[50] Neutrophils phagocytose dead tissue, protect against infection, and prepare the site for new tissue formation.[51] Within 48-72 hours, macrophages replace neutrophils, stimulating granulation and collagen formation.[52] However, prolonged inflammation can increase cytokine release, such as IL-1β, IL-6, and TNF-α, which further triggers proteolytic enzyme secretion and arachidonic acid metabolites, leading to tissue damage, delayed healing, and an increased risk of fibrosis and scarring.[53]

Flavonoids play a key role in reducing inflammation by inhibiting eicosanoid-producing enzymes such as phospholipase A2, lipoxygenase, and cyclooxygenase (COX), thereby lowering leukotriene and prostaglandin concentrations.[54] Additionally, flavonoids inhibit histamine release, protein kinases, phosphodiesterase, and transcriptase activation. More specifically, flavonoids reduce inflammatory mediators like prostaglandins, leukotrienes, and pro-inflammatory cytokines (IL-1β, TNF-α, IL-6, and IFN-γ), while increasing anti-inflammatory mediators such as IL-10. They also negatively regulate nuclear factor kappa B (NF-κB) expression and inhibit cyclooxygenase (COX) activity.[55]

Saponins exhibit corticomimetic activity, inhibit glucocorticoid degradation, and prevent the release of inflammatory mediators. Meanwhile, tannins function as astringents, disrupting microbial enzymes, shrinking skin pores, stopping bleeding, and accelerating wound closure.[54]

Oxidative Stress and Antioxidant Properties of Flavonoids and Tannins

Apart from inflammation, oxidative stress plays a critical role in wound progression. However, injuries, inflammation, or radiation exposure can increase reactive oxygen species (ROS) production, leading to oxidative stress and tissue damage. Excessive ROS levels prevent the wound from transitioning from the inflammatory to the proliferative phase, causing chronic inflammation and delayed healing.[56]

Managing oxidative stress can prevent burn wounds from deepening, making antioxidant therapy an essential option for reducing burn-related tissue damage.[57] Flavonoids exhibit antioxidant effects by scavenging free radicals, chelating catalytic metals, activating antioxidant enzymes, reducing alpha-tocopherol radicals, and inhibiting oxidase activity. Flavonoids also scavenge nitric oxide radicals when interacting with superoxide peroxynitrite radicals, preventing severe damage and inhibiting xanthine oxidation.[58]

Similarly, tannins possess strong antioxidant properties, neutralizing free radicals and protecting cells from oxidative damage. Research [59] confirms that 96% ethanolic extract of citrus mistletoe leaves has an IC50 value of <50 ppm, indicating very strong antioxidant activity.[60]

Antimicrobial Role of Flavonoids, Tannins, and Alkaloids

The inflammatory phase proceeds efficiently without infection, fibroblast proliferation will also occur optimally. Thus, infection is a major cause of burn wound healing failure, potentially leading

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to fatal outcomes. Under normal conditions, the immune system prevents infections by rapidly eliminating pathogens. However, if the immune system fails, infection can damage granulation tissue, growth factors, and the extracellular matrix, further hindering wound healing.

Flavonoids act as antimicrobial agents by suppressing nucleic acid synthesis, disrupting cell membrane function, and inhibiting bacterial energy metabolism. They also reduce adhesion and biofilm formation, increase membrane permeability, and inhibit bacterial growth. Tannins inhibit bacterial growth by chelating iron, disrupting cell wall synthesis, damaging bacterial membranes, and inhibiting fatty acid biosynthesis pathways. Meanwhile, alkaloids act as antimicrobials by interfering with peptidoglycan synthesis, preventing proper bacterial cell wall formation, ultimately leading to cell death.

Proliferation Phase and the Role of Bioactive Compounds

The proliferation phase begins after inflammation subsides, with fibroblasts appearing around day 3 and peaking by day 7 to form the extracellular matrix that fills the wound area. During this phase, macrophages produce growth factors such as PDGF and TGF-β.[61]

At this stage, wounds in test mice change from red in appearance to forming dry scabs or crusts. The proliferation phase is marked by granulation tissue formation and fibroblast activity, resulting in scab formation. The proliferation phase ends with the formation of new epithelial cells, collagen layers, and scab detachment, transitioning into the maturation phase.[62]

Saponins accelerate keratinocyte migration, which is essential for collagen synthesis and wound revitalization. Tannins promote angiogenesis by stimulating capillary formation and platelet-derived growth factors (PDGF and TGF-β), enhancing wound contraction and fibroblast activity. Meanwhile, flavonoids activate macrophages, which stimulate TGF-β secretion, supporting angiogenesis and fibroblast proliferation—key processes in collagen synthesis and tissue remodeling. Alkaloids also stimulate fibroblast synthesis, boosting collagen production.[63]

Maturation Phase and Final Tissue Remodeling

The maturation or remodeling phase is the final stage, refining tissue strength and quality. During this phase, immature collagen formed in the proliferation phase is remodeled into mature, structurally strong collagen via collagenase activity. This phase typically lasts several months, with wounds closing and hair regrowth occurring. Saponins enhance collagen synthesis through Smad 2 protein phosphorylation, supporting matrix regeneration at the wound site.[64]

CONCLUSION

This study demonstrates that the ethanol extract gel of citrus mistletoe leaves has potential as an effective alternative therapy for second-degree burn healing in white mice (*Mus musculus*). The physical quality tests showed that the gel had good stability, with an appropriate pH (pH 5), adequate homogeneity, and adhesion properties that met the standards. The highest wound healing percentage was observed in the gel with a 15% extract concentration, which closely matched the effectiveness of the positive control (Bioplacenton®). The presence of secondary metabolites in the extract, such as flavonoids, saponins, and tannins, contributed to the healing process through anti-inflammatory, antibacterial, antioxidant mechanisms, and enhanced collagen synthesis. Based on these findings, the ethanol extract gel of citrus mistletoe leaves can be considered a safe and cost-effective alternative for burn treatment, supporting the use of effective herbal medicine.

Conflict of Interest

The authors declare no conflict of interest.

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Authors' Declaration

The authors of this post attest to the originality of its material and accept full liability for any claims that may result from it.

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