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Innovative Gel Formulation with 70% Ethanol Extract of *Pandanus amaryllifolius* Leaf for Accelerated Healing of Second-Degree Burn in Rats

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ABSTRACT

Burns, particularly second-degree burns, are common injuries faced by many people. Pandan Wangi (*Pandanus amaryllifolius Roxb*), a plant known for its medicinal properties, has shown potential in treating burns. This study aimed to evaluate the potential of 70% ethanol leaf extract of *Pandanus amaryllifolius* gel formulation as a natural ingredient with strong wound-healing properties. *Pandanus amaryllifolius* leaf was extracted by maceration in 70% ethanol. The extract was subjected to qualitative phytochemical screening following standard procedures. Three gel formulations containing *Pandanus amaryllifolius* leaf extract at concentrations of 30% (F1), 35% (F2), and 40% (F3) were prepared using Viscolam MAC 10 as the gel base. The physicochemical properties (organoleptic, viscosity, pH, spreadability, and adhesion), irritation, and stability of the gel formulations were evaluated. The burn wound-healing activity of the gel formulations was evaluated in rats. The antibacterial activity of the gel formulations was evaluated against *Staphylococcus aureus* and *Pseudomonas aeruginosa* using the agar well diffusion method. Phytochemical screening of *Pandanus amaryllifolius* leaf extract revealed the presence of flavonoids, alkaloids, saponins, and tannins. The gel formulations demonstrated favorable physicochemical properties. Among the formulations, F3 containing 40% extract, showed the most rapid burn wound healing, reducing the recovery period by 9 days. F3 also demonstrated significant antibacterial activity against both *Staphylococcus aureus* and *Pseudomonas aeruginosa* with inhibition zone diameter of 6.0 and 3.8 mm, respectively. This study supports the potential of *Pandanus amaryllifolius* as a natural ingredient with strong wound-healing properties, further contributing to the development of herbal-based formulations for burn care.

Keywords: *Pandanus amaryllifolius Roxb*, Gel formulation, Second-degree burn, Antibacterial.

Introduction

Burn injuries, particularly second-degree, pose a significant medical challenge due to the ability to damage the epidermis and part of the dermis, leading to pain, inflammation, and the potential for serious complications if not properly managed. Effective burn wound healing requires rapid tissue regeneration, infection prevention, and inflammation control. Conventional treatments often comprise the use of synthetic drugs, which have proven effective but, in some cases, may lead to adverse effects such as delayed epithelialization or allergic reactions. With the increasing demand for safer and naturally derived therapies, the development of herbal-based formulations has become a major focus in pharmaceutical studies.[1–3]

One of the most promising natural ingredients is *Pandanus amaryllifolius*, commonly known as Pandan Wangi, which has been widely used in traditional medicine.

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Pandan leaf contain various bioactive compounds, including flavonoids, tannins, alkaloids, polyphenols, and saponins, which have antioxidant, anti-inflammatory, and antimicrobial properties, which can play a crucial role in accelerating burn wound healing. Several studies have investigated the pharmacological potential of *Pandanus amaryllifolius*, particularly in enhancing collagen synthesis, fibroblast proliferation, and angiogenesis, making it a strong candidate for innovative formulations in wound healing applications.[4,5] Previous studies have also evaluated the effects of various plant extracts, such as *Aloe vera*, *Centella asiatica*, and *Curcuma longa* on burn wound healing, and these extracts effectively accelerated wound recovery. However, the efficacy of *Pandanus amaryllifolius* in a topical gel formulation remains highly understudied.

Although, numerous formulations have used carbomer or hydroxypropyl methylcellulose (HPMC) as gelling agents, this study aimed to develop and evaluate a topical gel formulation using Viscolam® MAC, an innovative polymer known for its high stability, excellent spreadability, and controlled release of active compounds. The objective was to explore the potential of Viscolam® MAC as a promising alternative gelling agent to enhance the effectiveness of wound healing treatments. The novelty of this study lies in the use of *Pandanus amaryllifolius* extract, which is traditionally recognized for its antioxidant and anti-inflammatory properties but has not been extensively explored for topical wound healing applications. Furthermore, the combination of the extract with Viscolam® MAC-based gel represents a new approach to develop innovative wound care formulations with enhanced therapeutic efficacy.[6–8]

In this study, a 70% ethanol extract of *Pandanus amaryllifolius* leaf was incorporated into a gel formulation using Viscolam® MAC as the gelling agent. Ethanol (70%) was selected as the solvent due to the

optimal ability to extract both polar and non-polar bioactive compounds, thereby enhancing the therapeutic potential of the extract. The physicochemical properties of the gel, including viscosity, pH stability, homogeneity, and spreadability, were optimized to ensure effective skin absorption and sustained release of active compounds.[9] The effectiveness of the gel formulation was evaluated using *Mus musculus* models with second-degree burn injuries. *Mus musculus* is widely used in burn wound studies due to the skin structure and wound healing mechanisms, which closely resemble those of humans. The evaluation parameters included irritation testing and activity assessments to determine the gel impact on tissue regeneration. The obtained results were then compared to Bioplasenton, a well-established commercial product for burn treatment, which served as the positive control.[10–12]

One of the key advantages of this study is the comparative evaluation against Bioplasenton, a widely used product for burn wound treatment. Although, Bioplasenton is known to stimulate epithelialization and prevent infections, concerns have been raised regarding the potential for allergic reactions, antibiotic resistance, and limitations in long-term use. Given that the *Pandanus amaryllifolius* extract-based gel formulation has comparable or superior efficacy to Bioplasenton, it could serve as a safer, more affordable, and natural alternative for burn wound treatment.[13,14] The results have the potential to make a significant contribution to the development of herbal-based pharmaceutical products, offering an alternative to conventional synthetic therapies for burn wound. This innovative gel formulation could be further developed into a commercial product and explored for broader dermatological applications.[15,16] By integrating natural bioactive compounds, advanced polymer technology (Viscolam® MAC), and scientific validation, this study bridges the gap between traditional medicine and modern pharmaceutical innovation, with the potential to revolutionize herbal therapy for burn wound.

Materials and Methods

Chemicals and Equipment

The chemicals used include distilled water, 70% Ethanol (Jk Care®), DMDM Hydantoin (Raja Kimia®), Dragendorff reagent (Nitra Kimia®), $FeCl_3$ (Aloin®), Glycerin (Raja Kimia®), Propyleneglycol (Nitra Kimia®), Mayer's reagent (Aloin®), Liebermann-Burchard reagent (Aloin®), Triethanolamine (Nitra Kimia®), and Viscolam® MAC 10 (Nitra Kimia®). The equipment used include blender, climatic chamber, vacuum desiccator (Duran 30 cm®), homogenizer (HMG-6B®), caliper (taffware®), animal (KEMEI KM-1991®), scissors (KEMEI KM-1991®), electric soldering iron with a 20 mm diameter metal plate (taffware®), pH meter (Thermo Scientific®), stopwatch, (HAHNVAPOR®), viscometer Brookfield (NDJ-8S®, China), and analytical balance.

Plant collection and identification

Fresh leaves if *Pandanus amaryllifolius* were collected on January 11, 2024 from a local resident garden with the owner consent, located in Botupingge Village, Botupingge District, Bone Bolango Regency, Gorontalo, Indonesia (GPS coordinates: latitude 0.526685° N and longitude 123.098675° E). The plant material was identified by Abdullah Walangadi, a laboratory technician at the Pharmaceutical Biology Laboratory, Universitas Negeri Gorontalo.

Preparation of Pandanus amaryllifolius extract

The leaf samples were selected based on the criteria of having a dark green colour, being free from rot, and being undamaged. The selected leaves were washed with running water and air-dried until dried but not wilted. The dried leaf was cut into small pieces of approximately 1 cm and subsequently dried under indirect sunlight. Once completely dried, the leaves were ground using a blender, sieved through a 60-mesh sieve, and the weight of the dried powdered sample was recorded.[17] For the extraction process, *Pandanus amaryllifolius* leaf powder (500 g) was macerated in 70% ethanol (1000 mL) at room temperature. The mixture was shaken for 1 hour and then left for 3 × 24 hours with occasional shaking. The extract was filtered, and the resulting filtrate was evaporated on a water bath until a thick extract was obtained. The final extract was transferred into a glass bottle and stored in a refrigerator until further analysis and use.

Solvent-free extract test

The solvent-free extract test was conducted by adding two drops of concentrated H_2SO_4 and 1 mL of potassium dichromate to the extract. A colour change from orange to bluish-green indicates that the extract still contains ethanol.

Gel formulation

The gel formulation was developed incoporating 70% ethanol extract of *Pandanus amaryllifolius* leaves for wound healing applications, particularly burn injuries. The formulation was designed to ensure effectiveness, stability, and ease of application. Description: Table 1 shows the composition of gel formulations containing 70% ethanol extract of *Pandanus amaryllifolius* leaf. Three different formulations (F1, F2, F3) were prepared with varying concentrations of the extract: 30% for F1, 35% for F2, and 40% for F3. Each formula also contained 10% Viscolam MAC 10, 10% Propylene Glycol, 5% Glycerin, 0.1% DMDM Hydantoin, and 1% Triethanolamine. Distilled water was added to each formulation to complete the total volume to 100%. These formulations were tested for their effectiveness in burn wound healing. The extract concentration was varied in order to determine the optimal composition for maximum therapeutic efficacy.

Table 1: Composition of 70% ethanol leaf extract of *Pandanus amaryllifolius* gel formulations

Components	F1 (%)	F2 (%)	F3 (%)
70% Ethanol Extract of *Pandanus amaryllifolius* Leaf	30	35	40
Viscolam MAC 10	10	10	10
Propylene Glycol	10	10	10
Glycerin	5	5	5
DMDM Hydantoin	0.1	0.1	0.1
Triethanolamine	1	1	1
Distilled Water	Add to 100	Add to 100	Add to 100

F: Formula

Phytochemical screening of *Pandanus amaryllifolius leaf extract*

The phytochemical screening of *Pandanus amaryllifolius* leaf extract was conducted to determine the presence or absence of secondary metabolites such as alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, and phenolic compounds using standard qualitative tests. The alkaloid test was performed using Mayer and Dragendorff reagents, where the formation of a white or orange precipitate indicated a positive result.[18] The flavonoid test was carried out by adding magnesium powder and concentrated hydrochloric acid, resulting in a reddish or orange coloration for flavonoid presence.[19] The tannin test was performed with the addition of ferric chloride ($FeCl_3$) solution, which produced a blue-black or greenish coloration when tannins were

present.[20] The saponin test was conducted using a froth test, where the formation of stable foam upon vigorous shaking with water confirmed the presence of saponins.[21]

Protein target prediction

The potential of quercetin and kaempferol, flavonoid compounds in *Pandanus amaryllifolius* extract, as anti-inflammatory agents for burn wounds were analyzed through interaction with inflammatory target proteins (PDB: 1GQG and PDB: 5AV3). The analysis was conducted using an *in silico* approach with the assistance of SwissDock to evaluate the binding affinity of both flavonoids to proteins implicated in the inflammatory response. The ligands were downloaded from the website https://www.rcsb.org/structure/1GQG .

Organoleptic test of Pandanus amaryllifolius leaf extract and gel formulation

The organoleptic evaluation of *Pandanus amaryllifolius* leaf extract was conducted to assess the colour, odour, texture, and appearance using visual inspection and sensory analysis. The colour of the extract was observed under natural and artificial light to ensure uniformity and consistency. The odour was evaluated by direct inhalation to detect any characteristic or unusual scent. For the gel formulation, the texture and consistency were assessed by applying a small amount of the gel between the fingers to determine smoothness, homogeneity, and spreadability. Additionally, the appearance of the gel was examined for any signs of phase separation, precipitation, or air bubble formation, ensuring physical stability and aesthetic acceptability. The organoleptic evaluation results provide essential preliminary information regarding the consumer acceptability and quality of the gel formulation.[22–24]

Viscosity test of gel formulation

The viscosity of the gel formulation was measured to evaluate the flow properties, consistency, and ease of application. The measurement was conducted using a Brookfield viscometer NDJ-8S (Chongqing Drawell Instrument Co., Ltd, China) equipped with a spindle suitable for semi-solid formulations, with the gel sample maintained at 25°C ± 2°C to ensure consistency. Viscosity readings were taken at various rotational speeds (rpm) to assess the gel shear-thinning behavior, which is crucial for spreadability and application performance. The analysis was performed in triplicate, and the results were expressed in centipoise (cP). Viscosity data were compared with standard values for topical gel formulations to ensure appropriate rheological properties, contributing to product stability, ease of dispensing, and overall user acceptability. [25–27]

Stability test of gel formulation

The stability assessment of the gel formulation was conducted using temperature storage stability testing and cycling tests to evaluate the physical and chemical stability under varying conditions. For temperature storage testing, samples were stored at 4°C (refrigeration), 25°C (room temperature), and 40°C ± 2°C (accelerated conditions) for three months, with evaluations at 0, 7, 14, 30, 60, and 90 days for colour, odour, phase separation, pH, viscosity, and consistency changes. In the cycling test, the gel was subjected to six cycles of temperature fluctuations between 4°C and 40°C, each lasting 24 hours per temperature condition, followed by assessments of phase separation, precipitation, and viscosity (Chongqing Drawell Instrument Co., Ltd, China), and pH (Mettler Toledo, Europe). These tests help determine the stability, robustness, and shelf-life of the gel formulation, ensuring the quality, efficacy, and safety during storage and transportation.[28]

Irritation test of gel formulation

The irritation test was conducted to evaluate the dermal safety and tolerability of the gel formulation using healthy albino rats following OECD (The Organisation for Economic Co-operation and Development) guidelines for acute dermal irritation as previously described.[29,30] The test animals were acclimatized under controlled conditions of 22 ± 2°C, 50 ± 10% humidity, and a 12-hour light-dark cycle, with standard food and water *ad libitum*. A 4 cm² area of dorsal skin was shaved 24 hours before the test, and 0.5 g of the gel formulation was applied to the test site, with an untreated area serving as a negative control. The site was covered with a semi-occlusive dressing for 4 hours, and the residual gel was removed afterward. Observations were recorded at 1, 24, 48, and 72 hours post-application, assessing erythema, edema, and other skin reactions using the Draize scoring system (0 = no reaction, 4 = severe irritation). The study was performed in triplicate, with comparisons against a positive control (Bioplasenton gel).

Ethical approval

Ethical approval was obtained from the Health Research Ethics Committee of Gorontalo State University, Indonesia with ethi.cal approval number 048A/UN47.B7/KE/2024. The animals were handled humanely

Evaluation of the wound-healing activity of gel formulation

Experimental animals

The study was conducted using male albino rats 2-month-old weighing 150-200 g. The rats were acclimatized for three days before the experiment to ensure adaptation to the new environment and treatments. The rats were housed in standard cages under controlled conditions including temperature: 22 ± 2°C, humidity: 50 ± 10%, and a 12-hour light-dark cycle, with access to food and water *ad libitum*.[31,32]

Animal grouping

A total of 25 rats were used in this study, they were divided into five experimental groups, each consisting of five rats. The sample size calculation was based on the Federer formula :

$$(t-1).(n-1) \geq 15$$
$$(5-1).(n-1) \geq 15$$
$$4n-4 \geq 15$$
$$4n \geq 19$$
$$n \geq 4.5 \sim 5$$

Where:

• t = Number of experimental groups

• n = Sample size per group

Based on this calculation, the ideal sample size per group was determined to be five rats, with a total of 25 allocated across the five groups as follows:

Group I (Negative Control): Treated with gel base only

Group II (Positive Control): Treated with Bioplasenton gel

Group III: Treated with 30% *Pandanus amaryllifolius* extract gel

Group IV: Treated with 35% *Pandanus amaryllifolius* extract gel

Group V: Treated with 40% *Pandanus amaryllifolius* extract gel

Burn wound induction

Before burn wound induction, the dorsal fur of each rat was shaved using a sterile razor, and the exposed skin was gently cleaned with warm water to minimize skin damage and facilitate uniform burning. The burn wound was induced using a preheated metal plate (1.5 cm × 1.5 cm), heated over a Bunsen burner for approximately 1 minute, then applied to the dorsal skin of each rat for 5 seconds under mild anesthesia. Each rat received a single burn wound in a standardized location.[33,34]

Treatment administration

After burn wound induction, treatment was administered to each experimental group three times daily until complete wound healing was observed. The wound healing process was evaluated by measuring the wound diameter daily in each rat. The contraction rate and healing time were recorded to assess the efficacy of the formulations. Observations continued until complete wound closure was achieved.

Determination of antibacterial activity of gel formulation

The antibacterial activity of *Pandanus amaryllifolius* leaf extract gel was evaluated against *Staphylococcus aureus* (Gram-positive) and

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Pseudomonas aeruginosa (Gram-negative) using the agar well diffusion method as previously described.[35,36] Bacteria cultures were grown in Mueller-Hinton Broth (MHB) at 37°C for 24 hours, adjusted to 0.5 McFarland standard (1.5×10^8 CFU/mL), and spread onto Mueller-Hinton Agar (MHA) plates. Subsequently, sterile 6 mm wells were created in the agar, and 100 µL of the gel formulations at 30%, 35%, and 40% extract concentrations were applied, while the gel base (without extract) was used as a negative control. The plates were incubated at 37°C for 24 hours, after which the zones of inhibition (mm) were measured using a digital caliper Ultra Cal-V (PT. Metro Rekayasa Indonesia, Indonesia). The experiment was conducted in triplicate.

Results and Discussion

Phytochemical constituents of Pandanus amaryllifolius leaf extract

The phytochemical screening of *Pandanus amaryllifolius Roxb* leaf extract showed the presence of various bioactive compounds. The alkaloid test indicated a positive result with the formation of an orange colour and the flavonoid test showed a yellow colouration. The saponin test demonstrated the formation of foam that persisted for approximately 5 minutes, and the tannin test produced a dark bluish-black colouration, signifying a positive result. These results underscore the rich phytochemical profile of *Pandanus amaryllifolius* leaf, supporting its potential pharmacological applications. The phytochemical screening results summarized in Table 2 can be attributed to the specific chemical interactions between the bioactive compounds and the reagents used in the tests.

Table 2: Phytochemical constituents of 70% ethanol leaf extract *Pandanus amaryllifolius*

Compound	Test	Observation	Result
Alkaloid	Dragendorff	Orange coloured solution was formed	(+)
Flavonoid	0.1 mg powder Mg + 1 mL concentrated HCl	Yellow coloured solution was formed	(+)
Saponin	10 mL distilled water	Formation of foam which persisted for 5 minutes	(+)
Tannin	3 drops of 1% $FeCl_3$	Blackish blue solution was formed	(+)

+: Positive

The orange colouration in the alkaloid test occurs due to the formation of complexes between alkaloids and the Dragendorff reagent, which precipitate as orange salts. The yellow colour in the flavonoid test is a result of the reaction between flavonoids and magnesium in an acidic environment, leading to the formation of a flavonoid-metal complex. The foam formation is caused by the surfactant-like properties of saponins, which reduce surface tension, allowing the foam to persist for an extended period. Finally, the dark bluish-black colouration in the tannin test is due to the interaction between tannins and ferric ions (Fe^{3+}) in the $FeCl_3$ reagent, resulting in the formation of a stable ferric-tannin complex. These reactions are characteristic of the specific functional groups present in each class of secondary metabolites, thereby confirming the presence in the plant extract.[37]

Predicted protein target

Target prediction analysis results using Swissdock showed that Quercetin has significant potential as an anti-inflammatory agent evidenced by the interaction with multiple protein targets implicated in inflammatory pathways. Enzymes, constituting 40% of the predicted targets, play a crucial role in inflammatory responses, particularly cyclooxygenase-2 (COX-2), which is a primary target of non-steroidal anti-inflammatory drugs (NSAIDs). When quercetin effectively inhibits COX-2, it may exert a substantial anti-inflammatory effect. Additionally, proteases (20%), such as matrix metalloproteinases (MMPs), are implicated in tissue degradation and inflammation. Inhibition by quercetin could mitigate tissue damage associated with chronic inflammation. Quercetin also targets kinases (13.3%), including MAPK, JNK, and IKK, which regulate NF-κB signaling, a crucial pathway in cytokine production (TNF-α, IL-6, IL-1β). By inhibiting these kinases, quercetin could reduce inflammatory cytokine expression. Furthermore, the interaction with Family A G protein-coupled receptors (GPCRs) (6.7%) suggests a role in modulating immune responses, particularly those associated with histamine and prostaglandins. Quercetin also targets oxidoreductases and hydrolases (6.7%), which are closely associated with oxidative stress and chronic inflammation. By acting as an antioxidant, it may alleviate inflammation induced by reactive oxygen species (ROS). Finally, the association with primary active transporters (6.7%) may influence bioavailability and systemic distribution. These results support the hypothesis that quercetin inhibits key inflammatory pathways, including COX-2 and NF-κB, in a manner similar to betamethasone, thereby underscoring its potential as a natural anti-inflammatory agent (Figure 1).

A B

Figure 1: Protein target prediction with Swissdock simulation. (a) Quercetin molecular structure (b) Quercetin Predicted protein target represented in a pie chart. The chart categorizes the targets into different types, with the largest portion (40%) corresponding to enzymes. Other categories include primary active transporters, kinases, proteases, oxidoreductases, hydrolases, and family A G protein-coupled receptors, each contributing smaller percentages to the overall distribution

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Kaempferol has anti-inflammatory potential evidenced by the interaction with various protein targets implicated in inflammation and oxidative stress pathways. Although primary active transporters (20%) play a role in drug absorption and distribution rather than directly influencing inflammation, kaempferol interaction with proteases (13.3%), such as MMPs, suggests a role in mitigating tissue degradation and inflammatory processes. Enzymes (26.7%), particularly those related to COX-2 and lipoxygenase (LOX), indicate kaempferol potential to modulate inflammatory responses through reduction of prostaglandin and leukotriene synthesis. The presence of oxidoreductases (13.3%) among the targets further supports the anti-inflammatory mechanism, as these enzymes are associated with oxidative stress, which contributes to chronic inflammation. By interacting with oxidoreductases, kaempferol may help neutralize ROS, thereby reducing oxidative stress-induced inflammation.

Family A G protein-coupled receptors (GPCRs) (13.3%) are associated with inflammatory signaling, including histamine and prostaglandin pathways, suggesting that kaempferol could modulate immune responses and inflammatory cascades. Although hydrolases (6.7%) have a minor role in inflammation, the activity in biological molecule degradation may contribute to overall inflammatory regulation. Kaempferol targets kinases (6.7%) also play a significant role in inflammatory pathways such as NF-κB and MAPK, both of which are key regulators of cytokine expression and immune responses. These results suggest that kaempferol interaction with proteases, enzymes, oxidoreductases, GPCRs, and kinases may contribute to the anti-inflammatory properties, potentially making it a promising natural alternative for inflammation-related conditions (Figure 2).

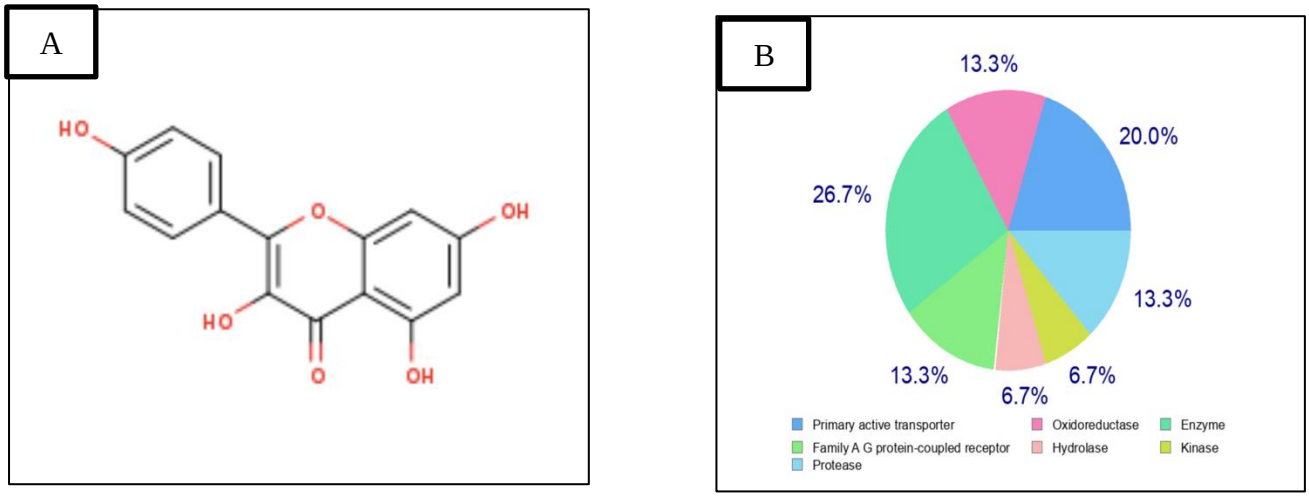

Figure 2: Protein target prediction with Swissdock simulation. (a) Kaempferol molecular structure, (b) Predicted protein targets of Kaempferol, shown in a pie chart. The largest portion (26.7%) is associated with primary active transporters, followed by enzyme targets (20.0%), and smaller contributions from proteases, oxidoreductases, kinases, hydrolases, and family A G protein-coupled receptors.

Organoleptic properties of *Pandanus amaryllifolius leaf extract*

The organoleptic evaluation of *Pandanus amaryllifolius* leaf extract showed characteristic properties. The extract had a dark greenish colour, attributed to the presence of chlorophyll and other plant pigments such as flavonoids and carotenoids extracted during the process. It also emitted a distinctive pandan-like aroma, primarily due to the compound 2-acetyl-1-pyrroline (2AP), a key aromatic component in *Pandanus amaryllifolius* leaf. Furthermore, the extract showed uniform miscibility, indicating a well-prepared and homogenized solution. This uniformity is likely due to the complete dissolution of bioactive compounds in the ethanol solvent, which efficiently extracts both polar and non-polar constituents. The organoleptic characteristics not only confirm the quality of the extract but also reflect the presence of phytochemicals that contribute to the therapeutic potential.[9,38]

Adhesion, spreadability, and viscosity of Pandanus amaryllifolius leaf gel formulations

The adhesion test was performed to determine the duration for which the gel formulations remained attached to the skin, which is crucial for enhancing the diffusion of active compounds and optimizing therapeutic effects. The results showed that Formula I (F1) adhered for 6 seconds, Formula II (F2) for 8 seconds, and Formula III (F3) for 7 seconds. These values indicated that all formulations met the criteria for good adhesion, ensuring sufficient contact time for the active ingredients to penetrate the skin. The differences in adhesion duration may be attributed to variations in viscosity and extract concentration. F 2, having the longest adhesion time, is likely to possess an optimal balance between gel base components and extract concentration. Despite slight variations, all three formulations showed acceptable adhesion properties, suitable for topical application[9] (Table 3). The spreadability test results further confirmed the usability of the formulations. F1 and F2 had a spread area of 5 cm, while F3 showed slightly greater spreadability at 5.5 cm. This variation may be influenced by differences in viscosity and gel consistency, but all formulations maintained an appropriate spreadability range, allowing for easy application and effective coverage of the affected area. The combination of adequate adhesion and spreadability ensures that the 70% ethanol extract gel of *Pandanus amaryllifolius* possesses favourable physical characteristics, making it a promising formulation for burn wound healing applications.[9]

Table 3: Adhesion and spreadability of 70% ethanol leaf extract of *Pandanus amaryllifolius* gel formulations

Formula	Adhesion	Spreadability
	Time (Second)	Area (cm^2)
F1	6	5
F2	8	5
F3	7	5.5

The viscosity of the *Pandanus amaryllifolius* leaf gel formulations was measured using a Brookfield viscometer to evaluate the initial rheological properties. All formulations used Viscolam MAC as the gelling agent at a consistent concentration of 10%, with variations only in the active ingredient content. Formula 1 (F 1) contained 30% pandan leaf extract, while Formula 2 (F2) and 3 (F3) contained 35% and 40% respectively. The initial viscosity results showed a decreasing trend with higher active ingredient concentrations, where F I had the highest viscosity at 9500 cP, F 2 at 9640 cP, and F 3 at 8400 cP on day 0. Over a period of 7 days, the viscosity of all formulations gradually declined, with F1, F2, and F3 stabilizing at 6020 cP, 5920 cP, and 5130 cP, respectively. This decline in viscosity may be attributed to the interaction between the extract components and the gel matrix, which potentially impacts the network stability. The differences in initial viscosity are likely influenced by the concentration of active compounds, with higher extract levels (F3) leading to a less structured gel network. Subsequent testing, such as cycling tests, will further evaluate the influence of temperature variations on the stability and viscosity of the gel formulations. These results will provide a deeper understanding of the gel behaviour under different environmental conditions, crucial for ensuring product stability and efficacy[39] (Table 4).

Table 4: Viscosity of 70% ethanol leaf extract of *Pandanus amaryllifolius* gel formulations

Day	Viscocity (cP)		
	F 1	**F 2**	**F 3**
0	9500	9640	8400
1	8830	9000	7290
2	7940	8350	6880
3	7850	7580	6160
4	6950	7030	6000
5	6320	6820	5980
6	6100	6250	5770
7	6020	5920	5130

Stability of Pandanus amaryllifolius leaf gel formulations
pH stability
The pH cycling test was conducted to evaluate the stability of the gel formulations under alternating temperature conditions using a Climatic Chamber. Each cycle consisted of 48 hours, comprising 24 hours at 4°C followed by 24 hours at 40°C. The gel formulations used Viscolam MAC at a 10% concentration as the gelling agent, while the active ingredient concentration varied including 30% *Pandanus amaryllifolius* leaf extract for Formula 1 (F1), 35% for Formula 2 (F2), and 40% for Formula 3 (F3).
The pH results showed consistent stability across all cycles for the formulations. F1 had a gradual increase in pH, from 6.3 (Cycle 1) to 6.6 (Cycle 6) at both temperatures. Similarly, F2 showed a slight increase from 6.4 (Cycle 1) to 6.7 (Cycle 6), and F3 had stable pH values increasing from 6.5 (Cycle 1) to 6.7 (Cycle 6) under the same conditions. This pH stability can be attributed to the properties of Viscolam MAC as a gelling agent, which is highly resistant to temperature variations. The polymer structure protects against physicochemical changes caused by extreme temperatures, thereby preserving the gel matrix integrity and preventing chemical reactions that could affect pH[40,41] (Table 5 and Figure 3).
The *Pandanus amaryllifolius* leaf extract used contains stable phytochemical compounds such as flavonoids and tannins, which have antioxidant properties and are resistant to degradation within the tested temperature range. These factors, combined with the optimized formulation, contribute to the gel ability to maintain pH stability despite exposure to varying temperatures. The minimal pH changes during the cycling test demonstrate that these formulations are suitable for use and storage under diverse environmental conditions.[42,43]

Table 5: pH stability of 70% ethanol leaf extract of *Pandanus amaryllifolius* gel formulations

Cycle	pH					
	F1 (40°C)	**F2 (40°C)**	**F3 (40°C)**	**F1 (4°C)**	**F2 (4°C)**	**F3 (4°C)**
1	6.3	6.4	6.5	6.3	6.4	6.5
2	6.3	6.5	6.5	6.4	6.4	6.5
3	6.4	6.6	6.6	6.4	6.5	6.6
4	6.4	6.6	6.6	6.5	6.6	6.7
5	6.5	6.7	6.7	6.6	6.6	6.7
6	6.6	6.7	6.7	6.7	6.7	6.8

Viscosity stability
The results showed an overall increase in viscosity across all formulations during the cycling test. For instance, F1 had a viscosity range from 6020 cP (Cycle 1) to 6541 cP (Cycle 6) at 40°C, and from 6020 cP (Cycle 1) to 7473 cP (Cycle 6) at 4°C. Similarly, F2 showed an increase from 5920 cP (Cycle 1) to 7440 cP (Cycle 6) at 40°C, and from 5920 cP (Cycle 1) to 7860 cP (Cycle 6) at 4°C. F3, with the highest concentration of active extract, demonstrated an increase in viscosity from 5130 cP (Cycle 1) to 6964 cP (Cycle 6) at 40°C, and from 5130 cP (Cycle 1) to 6960 cP (Cycle 6) at 4°C (Table 6 and Figure 4).
The observed increase in viscosity across cycles indicates that the formulations became slightly more structured over time, possibly due to further polymer-extract interactions and the effect of temperature fluctuations. The stability of viscosity suggests that Viscolam MAC effectively maintained the gel network integrity under varying thermal conditions, regardless of the concentration of active ingredients. These results underscore the robustness of the gel formulations for storage and application in diverse environmental conditions.[39]

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Table 6: Viscosity of three gel formulations (F1, F2, F3) at 40°C and 4°C during a cycling test

Cycle	Viscosity					
	F1 (40°C)	F2 (40°C)	F3 (40°C)	F1 (4°C)	F2 (4°C)	F3 (4°C)
1	6020	5920	5130	6020	5920	5130
2	6040	6220	5170	6030	6012	5240
3	6756	6830	5420	6979	6750	5840
4	6783	7120	5582	6930	6873	5962
5	6662	7250	6675	7282	7652	6350
6	6541	7440	6964	7473	7860	6960

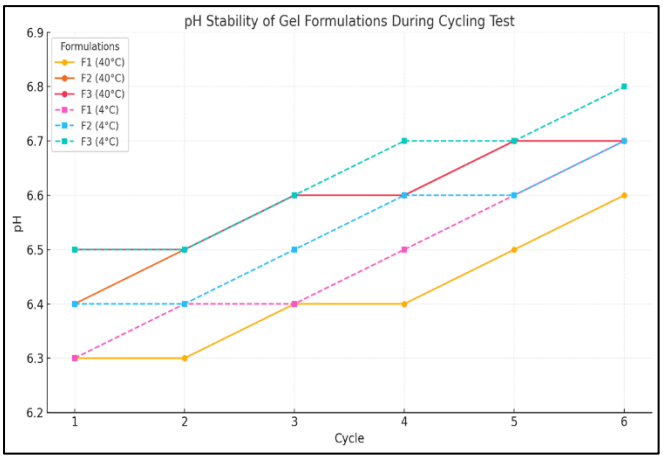

Figure 3: pH stability of gel formulations (F1, F2, F3) during a cycling test at two different temperatures: 40°C and 4°C. The graph illustrates that all formulations showed a gradual increase in pH over the cycles, with formulations at 40°C exhibiting a more noticeable rise in pH compared to those stored at 4°C. F1, F2, and F3 at 40°C demonstrated a steady increase, while formulations at 4°C remained relatively stable but with slight upward trends

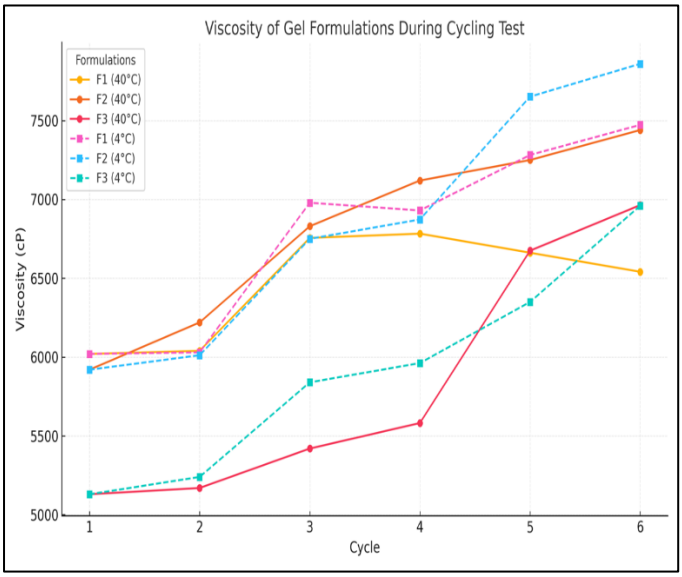

Figure 4: Viscosity stability of gel formulations (F1, F2, F3) during a cycling test at 40°C and 4°C. At 40°C, viscosity increased over the cycles, with F2 showing the largest change. Formulations at 4°C remained stable with minimal changes in viscosity.

Irritant effect of Pandanus amaryllifolius leaf gel formulations

The irritation test was conducted on rats to evaluate the dermal safety of *Pandanus amaryllifolius* leaf gel formulations. The gel was applied to the dorsal area, and observations were made at 24 hours, 48 hours, and 72 hours post-application. The results showed no signs of irritation, as indicated by the absence of erythema and edema across all formulas. The irritation scores for all observations were consistently 0, which falls below the irritation threshold, where scores between 1 and 4 indicate varying levels of irritation severity (Table 7 and Figure 5).

The absence of irritation can be attributed to several factors. First, the use of Viscolam MAC as the gelling agent plays a significant role in maintaining the skin compatibility of the formulation. Viscolam MAC is known for biocompatibility and widely used in dermatological formulations due to the gentle nature and low potential to cause irritation. Second, the 70% ethanol extract of *Pandanus amaryllifolius* leaf likely contains stable bioactive compounds, such as flavonoids and tannins, that have anti-inflammatory and soothing properties. These compounds may help reduce the risk of skin irritation by mitigating any inflammatory responses.[44]

The optimized formulation also ensures that the pH of the gel is close to the natural pH, which minimizes the risk of disrupting the skin barrier. The results confirm that the *Pandanus amaryllifolius* leaf gel formulations are safe for topical application and unlikely to cause irritation, suggesting suitability for further development and use in clinical or commercial settings.

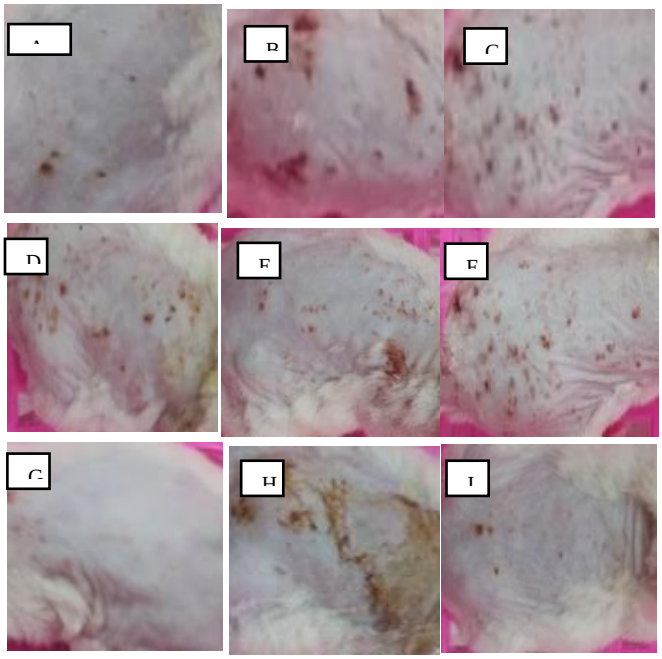

Figure 5: Irritation of *Pandanus amaryllifolius* leaf extract gel. A, B, C) F1 with 24 hours observation, D, E, F) F2 with 48 hours observation, G, H, I) F3 with 24 hours observation

All the images reveal no visible irritation signs, confirming the absence of erythema or edema in the test areas

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Table 7: Irritation test of gel formulation with 70% ethanol leaf extract of *Pandanus amaryllifolius*

Formula	Observation time					
	24 hours		48 hours		72 hours	
	Erythema	Edema	Erythema	Edema	Erythema	Edema
F1	0	0	0	0	0	0
F2	0	0	0	0	0	0
F3	0	0	0	0	0	0
Total	0	0	0	0	0	0
Clasification	No irritation					

Burn wound healing properties of Pandanus amaryllifolius leaf gel formulations

Pandanus amaryllifolius leaf extract gel formulations were shown to be effective in promoting burn wound healing, measured in millimeters over 18 days. This study compared the performance of three gel formulations with varying extract concentrations (30%, 35%, and 40%) against a commercial control (Bioplacenton) and an untreated group. The untreated group had the slowest wound healing process, with the wound size reducing from an average of 15.2 mm on Day 1 to 0 mm on Day 18, reflecting the natural healing progression without external aid (Table 8 and Figure 6).

In comparison, the Bioplacenton-treated group showed a faster healing rate, with wounds healing completely by Day 15. The 30% and 35% pandan extract gel formulations demonstrated comparable results to Bioplacenton, achieving full wound closure by Day 15. The 40% extract gel showed the fastest healing, with the wound size reducing to 0 mm by Day 12, outperforming both the Bioplacenton and other formulations. This suggests a dose-dependent relationship, where higher concentrations of pandan extract led to more rapid healing.[14,16]

The enhanced healing observed in the *Pandanus amaryllifolius* gel formulations, especially the 40% extract formulation, can be attributed to the bioactive compounds such as flavonoids, tannins, and polyphenols. These compounds have strong antioxidant, anti-inflammatory, and antimicrobial properties, which collectively promote fibroblast proliferation, collagen synthesis, and tissue regeneration while mitigating oxidative stress. The results demonstrate the potential of *Pandanus amaryllifolius* leaf extract gel, particularly at higher concentrations, as a natural and effective alternative to conventional burn wound treatments.[45]

Table 8: Burn wound healing activity of 70% ethanol leaf extract of *Pandanus amaryllifolius* gel formulation

No.	Group	Replicate	Wound size (mm)						
			Day 1	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18
1	No Treatment	1	15.1	13.2	11.3	9.8	6.8	3.5	0
		2	15.3	13.1	11.0	9.7	6.7	3.3	0
		3	15.2	13.2	11.2	9.8	6.9	3.6	0
2	Bioplacenton	1	15.1	13.6	10.6	7.5	4.6	1.1	0
		2	15.1	13.7	10.8	7.7	4.8	0.6	0
		3	15.1	13.5	10.4	7.4	4.4	0	0
3	Gel Extract 30%	1	15.0	12.2	9.0	6.5	2.9	0	0
		2	15.0	12.3	9.1	6.6	2.9	0	0
		3	15.0	12.0	8.9	6.3	2.5	0	0
4	Gel Extract 35%	1	15.0	12.5	7.2	6.5	2.9	0	0
		2	15.0	12.8	7.5	6.6	2.9	0	0
		3	15.0	12.8	7.7	6.3	2.5	0	0
5	Gel Extract 40%	1	15.0	11.0	6.6	0.9	0	0	0
		2	15.0	11.2	6.5	0.7	0	0	0
		3	15.0	12.5	7.6	1.5	0	0	0

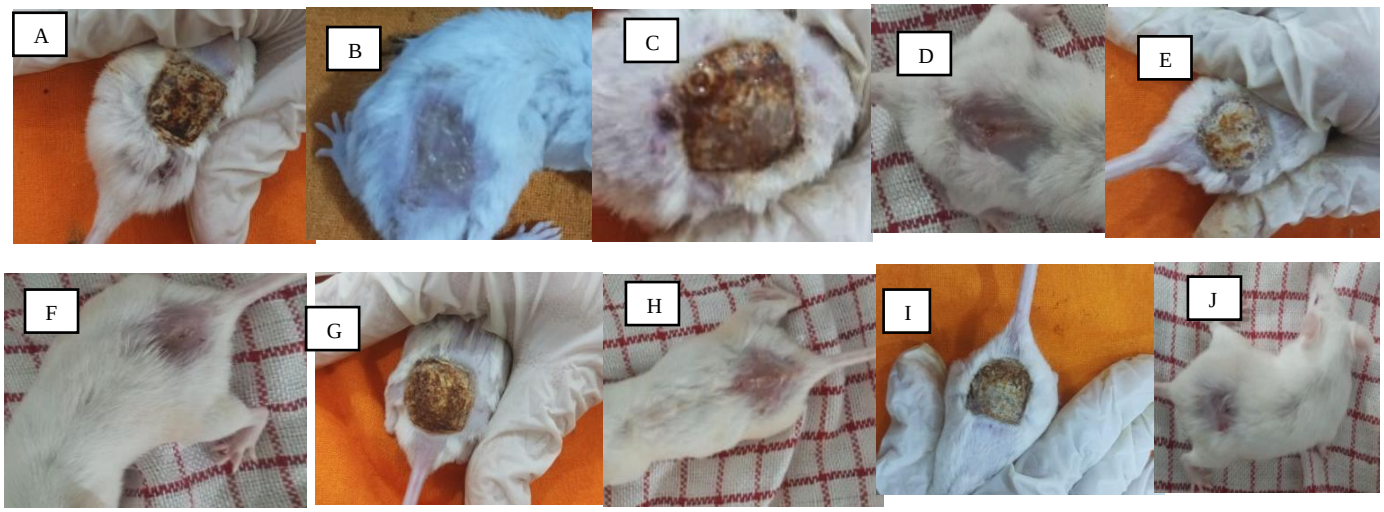

Figure 6: Burn wound healing activity of *Pandanus amaryllifolius* leaf extract gel. (A) No treatment day 1, (B) No treatment day 15, (C) Bioplacenton day 1, (D) Bioplacenton day 15, (E) F1 (30% extract) day 1, (F) F1 (30% extract) day 12, (G) F2 (35% extract) day 1, (H) F2 (35% extract) day 12, (I) F2 (40% extract) day 1, (J) F2 (40% extract) day 9.

Antibacterial activity of Pandanus amaryllifolius leaf gel formulations

The antibacterial activity test of *Pandanus amaryllifolius* leaf gel was conducted against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, two bacteria commonly associated with wound infections, particularly in burn. The results showed that the gel demonstrated significant inhibitory effects, with an average inhibition zone of 6 mm for *Staphylococcus aureus* and 3.8 mm for *Pseudomonas aeruginosa*. This indicates that the gel possesses effective antibacterial properties, particularly against *Staphylococcus aureus*, known to be a major cause of skin and soft tissue infections. The smaller inhibition zone for *Pseudomonas aeruginosa*, a Gram-negative bacteria with a more robust outer membrane, suggests that higher concentrations of the extract or additional antimicrobial agents might be needed for improved activity[46] (Figure 7).

The antibacterial efficacy of the gel can be attributed to the rich content of bioactive compounds such as flavonoids, tannins, and saponins, known for antimicrobial properties. Flavonoids and tannins disrupt bacteria cell membranes and interfere with protein synthesis, while saponins act as natural detergents that destabilize microbial membranes. The gel ethanol-based extraction process also enhances the solubility and bioavailability of the compounds, further contributing to antibacterial action. These results suggest that *Pandanus amaryllifolius* leaf gel has potential as a natural alternative to combat bacterial infections in burn wounds, reducing the risk of complications and promoting better healing outcomes.

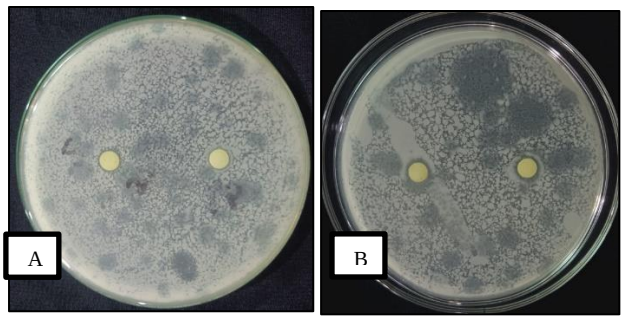

Figure 7: Antibacterial activity of *Pandanus amaryllifolius* leaf extract gel. (A) Inhibiton zone 6 mm *Staphylococcus aureus*, (B) Inhibiton zone 3.8 mm *Pseudomonas aeruginosa*

Conclusion

The findings from this study show that the 70% ethanol extract gel formulation of *Pandanus amaryllifolius* leaf demonstrated favorable physical properties, attributed to the use of Viscolam MAC as a gelling agent, which provided stability and consistency to the gel matrix. The irritation test confirmed that the gel is safe for topical application, showing no signs of erythema or edema across all formulations. Furthermore, the burn wound healing efficacy of the gel was significant, with faster recovery rates compared to the control, emphasizing the potential as an effective wound treatment. The antibacterial activity tests also indicated promising results, with significant inhibition zones against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, two common pathogens in wound infections. These results collectively suggest that the leaf gel is a safe, effective, and promising natural alternative for managing burn wounds and preventing bacteria infections. Additionally, this study supports the potential of *Pandanus amaryllifolius* as a natural medicinal ingredient with strong wound-healing properties, further contributing to the development of herbal-based formulations for burn care. Combining the extract with other synergistic herbal agents could potentially broaden the therapeutic spectrum, making it a valuable component in integrated wound care systems.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and any liability for claims relating to the content of this article will be borne by them.

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