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FORMULATION OF GEL EXTRACT OF GAMAT LEAF (TINOMISCIUM PETIOLARE HOOK.F & THOMSON) IN HEALING OF DIABETIC ULCERS AND BACTERIAL INHIBITORY POWER IN RATS

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ABSTRACT
Diabetic foot ulcers (DFUs) present a critical clinical challenge, where persistent bacterial infections frequently stall the healing cascade and escalate amputation risks. This delayed recovery is worsened by growing antibiotic resistance against conventional topical agents, underlining an urgent demand for multi target, plant derived alternatives. Although Tinomiscium petiolare Hook.f. & Thomson (locally known as Gamat leaf) is rich in tissue regenerative and antimicrobial compounds, its therapeutic potential in chronic diabetic wound microenvironments has not yet been investigated. This study aimed to formulate Gamat leaf extract (Tinomiscium petiolare) into a gel preparation and evaluate its effectiveness on diabetic ulcer healing and antibacterial inhibitory power in vivo using diabetic rat models. Method: This experimental study included the extraction of Gamat leaves by maceration with 96% ethanol, followed by gel formulation with varying extract concentrations (F1, F2, F3). The bacterial inhibition test was conducted in vitro (well method). The in vivo test used male Wistar rats induced by streptozotocin (STZ) to achieve a diabetic condition, then an excision wound was made as an ulcer model. The parameters observed included physical evaluation of the gel, percentage of wound area shrinkage over 14 days, and bacterial inhibition zone. GC-MS analysis identified dominant bioactive compounds with anti-inflammatory and antioxidant properties. The preparation showed stable viscosity, ensuring optimal adhesion and active ingredient penetration. HE results demonstrated significant increases in collagen deposition and epithelialization compared to the control group. The synergy of chemical, physical, and biological parameters proves that Gamat Leaf is a strong candidate for standardized pharmaceutical ingredients in accelerating wound healing.

Keywords: gamat leaf; GC-MS; histopathology; viscosity; wound healing

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INTRODUCTION

Diabetic ulcers occur in approximately 15–25% of patients with Diabetes Mellitus, with an annual incidence exceeding 2%, and affect about 5–7.5% of patients with neuropathy. According to the World Health Organization (WHO), the highest prevalence of Diabetes Mellitus is observed in individuals aged over 30 years, reaching 10.8%. The risk of amputation in patients with diabetes is 15–46 times higher than in non-diabetic individuals, with a post-amputation mortality rate of 15.89%, and the prevalence of diabetic ulcers reported at 9.4% (WHO) (Iversen et al., 2009; Ong et al., 2023; P. Zhang et al., 2017). In Indonesia, the prevalence of diabetic ulcers is approximately 15%, with an amputation rate of 30%. In addition, the one-year mortality rate after amputation reaches 14.8%. Notably, the number of patients with diabetic ulcers in Indonesia has increased by approximately 11%(Hariftyani et al., 2021).(Bhardwaj et al., 2020).

Tinomiscium petiolare Hook.f. & Thomson is a plant species with high diversity distributed across tropical Asian countries, including Vietnam (Bhardwaj et al., 2020). This species naturally grows in forest ecosystems with temperate and humid biomes at altitudes ranging from 200 to 600 m above

sea level ((Dembitsky, 2023). In traditional medicine, various parts of T. petiolare have been used to treat diseases due to its medicinal properties. In Malaysia, the roots of T. petiolare are used to relieve rheumatism and colds, while the sap is used to reduce fever. In Indonesia, the leaves of T. petiolare are traditionally used to treat severe wounds. In the Philippines, diluted milky white sap is used as an eye wash, whereas in Vietnam, the sap of T. petiolare is utilized to prevent tooth decay and as an antifungal agent ((Bhardwaj et al., 2020).

In Indonesia, gamat is known to have various medicinal uses. Among the Dayak Danum and Ngaju communities, gamat is utilized as an aphrodisiac, contraceptive, and to enhance stamina after childbirth. In Java, its roots and stems are traded under the name “kayu seriawan” and are used to alleviate fever and oral ulcers ((Cahyono et al., 2020). Phytochemical screening has demonstrated that gamat contains secondary metabolites, including flavonoids, alkaloids, triterpenes, anthrones, steroids, and essential oils. Previous studies on medicinal plants containing similar secondary metabolites have shown their ability to regulate cytokines such as IL-1β, TNF-α, and various growth factors involved in the wound healing process ((Hassan Abdel-Rhman et al., 2015; Purushothaman et al., 2025)). During the inflammatory phase, prostaglandins, leukotrienes, and cytokines (TNF-α) are produced, leading to vasodilation and increased blood flow, including the recruitment of neutrophils to the wound site. In the proliferative phase, wound healing is naturally stimulated by IL-1β, platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF), which contribute to proteoglycan and collagen formation ((Toy et al., 2015)).

To address this gap, this study was designed to formulate a topical hydrogel incorporating *Tinomiscium petiolare Hook.f. & Thomson* (Gamat leaf) extract and systematically evaluate its therapeutic efficacy. The research focuses on assessing the gel's physicochemical properties, particularly its viscosity, alongside it is in vitro bacterial inhibitory power. Furthermore, we investigated in vivo wound healing acceleration and histopathological changes in an induced diabetic ulcer rat model, substantiated by GC-MS analysis to profile the underlying bioactive compounds

METHOD

Material used in this research as Gamat leaves, ethanol 96%, Carbopol, Triethanolamine, Nipagin, Carbopol, Glycerin, Aquadest, ciprofloxacin, and povidone iodin ointment as a positive control. An instrument used in this research are maceration equipment consisting of a bottle maceration, a beaker glass, a flannel, mesh test sieve 20, alluminium foil, a funnel, an oven, stems the mixer, a weight, moisture ballance, the evaporators, tweezers, pipet drops, paper weigh, filter paper, vial, rat home, xylazine, scalpel, spuit, the glass and needle. In this study the experimental animal used a male white rat with a wistar strain (Ratus Novergicus) age 16 weeks at a weight of 150 to 250 grams. The number of rats used by 16 and grouped into 4 groups of treatment with groups of 2 rats. Previously on rat were aclimatized for seven days. Before being used for research, Animals tried to be fasting 18 hours but were still given a drink.

Generation of ethanol extracts of Gamat leaf

Leaf dust weighed a thousand grams, inserted in a dark - colored container, added 7,5 litres of ethanol 96 %. Then stir the dust and closed immediately, it was kept in a room that was spared the sun's rays and held for 72 hours, with occasional snitching. After 72 hours, then filtered with flannel fabric, filtrate is held in a glass beamer and rounded up using rotary evaporator. Extract results used for ethanol-free testing and extract rendement calculations. Processing of gamat plant materials began with submitting the samples for phytochemical analysis. After obtaining the HPLC results, gamat extract was prepared using the maceration technique with 96% ethanol as the solvent. Subsequently, a gel formulation containing gamat leaf extract was developed.

The preparation of the test animals

A total of 16 rats were utilized in this study following an initial acclimatization period. Diabetes

was induced through the administration of Streptozotocin (STZ), with blood glucose levels monitored subsequently. Only rats exhibiting a blood glucose concentration exceeding 200 mg/dL were included in the experimental trials. The subjects were subsequently randomly assigned into four treatment groups, with each group consisting of four rats. Grouping of test animals as follows:

T1: Diabetic ulcer rat group treated with 10% povidone iodine ointment

T2: Diabetic ulcer rat group treated with 5% gamat leaf extract gel

T3: Diabetic ulcer rat group treated with 10% gamat leaf extract gel

T4: Diabetic ulcer rat group treated with 15% gamat leaf extract gel

RESULT

GC-MS Profile of Tinomiscium petiolare leaf Extract

The chemical composition of the ethanolic extract of Gamat leaves (Tinomiscium petiolare Hook.f & Thomson) was characterized using Gas Chromatography-Mass Spectrometry (GC-MS). The chromatogram revealed several prominent peaks, representing various bioactive secondary metabolites. The identified compounds, along with their retention times (RT) and peak area percentages (%), are summarized in Table 1.

Tabel 1.
Phytochemical constituents of Gamat leaf extract identified by GC-MS

Molecular formula	Compound name	Chemical class	Biological Activity	Peak Area (%)
$C_{10}H_{20}O_2$	Decanoic acid (CAS) Capric acid	primary metabolites	Anticonvulsant, antimicrobial	1.21
$C_{12}H_{24}O_2$	Dodecanoic acid (CAS) Lauric acid	Primary metabolites	Antimicrobial	4.39
$C_{20}H_{38}$	Neophytadiene	Diterpene	Antimicrobial Anti-inflammatory Antioxidant Anticancer	1.51
$C_{20}H_{38}$	Neophytadiene	Diterpene	Antimicrobial Anti-inflammatory Antioxidant Anticancer	0.53
$C_{15}H_{28}O$	2,10-Dodecadien-1-ol, 3,7,11-trimethyl-, (Z)- (CAS) DL-6,7-DIHYDRO-2,CIS-FARNESOL	sesquiterpenes	Antimicrobial	0.43
$C_{23}H_{46}O$	12-Tricosanone (CAS) Lauron	Aliphatic ketone	Antimicrobial	0.74
$C_{23}H_{46}O$	12-Tricosanone (CAS) Lauron	Aliphatic ketone	Antimicrobial	1.44
$C_{16}H_{32}O$	Oxirane, tetradecyl-	Epoxide	Antimicrobial	0.25
$C_{16}H_{32}O$	Oxirane, tetradecyl-	Epoxide	Antimicrobial	2.02
$C_{30}H_{50}$	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl- (CAS) Squalene	Triterpenes	Antioxidant, cardioprotective	0.91
$C_{30}H_{50}$	2,6,10,15,19,23-HEXAMETHYL-2,6,10,14,18,22,-TETRACOSAHEXAENE	Triterpenes	Antioxidant, cardioprotective	2.50
$C_{30}H_{50}$	2,6,10,15,19,23-HEXAMETHYL-2,6,10,14,18,22,-TETRACOSAHEXAENE	Triterpenes	Antioxidant, cardioprotective	2.10
$C_{30}H_{50}$	2,6,10,15,19,23-HEXAMETHYL-2,6,10,14,18,22,-TETRACOSAHEXAENE	Triterpenes	Antioxidant,	4.28
$C_{20}H_{40}O$	:2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (CAS) Phytol	Diterpene	Antimicrobial, Anti-inflammatory, antioxidant, anticancer	5.48
$C_{20}H_{40}$	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	Diterpene	Anti-inflammatory, antioxidant, anticancer	0.35
$C_{18}H_{34}O_2$	9-Octadecenoic acid (Z)- (CAS) Oleic acid	Primary metabolites	Anti-inflammatory	4.65
$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester (CAS) Methyl	fatty acid	Anti-inflammatory,	0.94

Molecular formula	Compound name	Chemical class	Biological Activity	Peak Area (%)
	palmitate	methyl ester	antioxidant, anticancer	
$C_{19}H_{36}O_2$	9-Octadecenoic acid (Z)-, methyl ester (CAS) Methyl oleate	fatty acid methyl ester	Anti-inflammatory, antioxidant, anticancer	0.66
$C_{23}H_{46}O$	12-Tricosanone (CAS) Lauron	Aliphatic ketone	Antimicrobial, Anti-inflammatory	1.13
$C_{23}H_{46}O$	12-Tricosanone (CAS) Lauron	Aliphatic ketone	Antimicrobial, Anti-inflammatory	0.64
$C_{18}H_{36}O$	Oxirane, hexadecyl-	Epoxide	Antimicrobial, Anti-inflammatory	2.25
$C_{28}H_{48}O_2$	Gamma.-Tocopherol	Phytosterols	Anti-inflammatory, antioxidant, anticancer	1.13
$C_{29}H_{48}O$	4-Stigmasten-3-one, Sitostenone	Phytosterols	Antimicrobial	1.22
$C_{29}H_{48}O$	4-Stigmasten-3-one, Sitostenone	Phytosterols	Antimicrobial	0.43
$C_{28}H_{44}O$	Ergosta-4,22-dien-3-one (CAS) ERGOST-4,5-22,23-DIENE-3-ONE	Phytosterols	Antimicrobial, Anti-inflammatory	0.66
$C_{20}H_{32}$	(E,E)-7,11,15-Trimethyl-3-methylene-hexadeca-1,6,10,14-tetraene	Diterpene	Antimicrobial	0.50
$C_{31}H_{62}O$	16-Hentriacontanone (CAS) Palmitone	Ketones	Antimicrobial	2.87
$C_{31}H_{62}O$	16-Hentriacontanone (CAS) Palmitone	Ketones	Antimicrobial	2.07
$C_{30}H_{52}O_2$	TRICYCLO[20.8.0.0E7,16]TRIACONTAN, 1(22),7(16)-DIEPOXY-	Triterpenoid	Antimicrobial	0.47
$C_{15}H_{26}O$	FARNESOL ISOMER A	Sesquiterpene terpenoids	Antimicrobial	6.87
$C_{18}H_{34}O$	9-Octadecenal, (Z)- (CAS) CIS-OCTADEC-9-ENAL	Aldehyde	Antimicrobial, antioxidant	11.01
$C_{18}H_{34}O$	9-Octadecenal, (Z)- (CAS) CIS-OCTADEC-9-ENAL	Aldehyde	Antimicrobial, antioxidant	0.60

Evidence from GC-MS characterization points to a rich composition of terpenoids and aliphatic derivatives within Gamat leaves. This unique chemical signature is likely responsible for the extract's ability to accelerate tissue repair and overcome the complications of diabetic wound recovery.

Physical Evaluation Results of Gamat Leaf Extract Gel Formulations

The results of the physical property evaluations for the Gamat leaf extract gel formulations (F1, F2, and F3) are summarized in Table 2. The parameters assessed include organoleptic observations, homogeneity, pH value, spreadability, adhesiveness, and viscosity to ensure the stability and safety of the preparations for topical application.

Tabel 2. Physical Evaluation Results of Gamat Leaf Extract Gel Formulations

Parameters	F1 (5% Extract)	F2 (10% Extract)	F3 (15% Extract)	Specification/ Standards
Organoleptic	Ligth green, Characteristic odor	Green, Characteristic odor	Dark green, Characteristic odor	Homogeneous, smooth texture
Homogeneity	Homogeneous	Homogeneous	Homogeneous	No coarse particles
pH	6.11 ± 0.08	5.80 ± 0.03	5.51 ± 0.11	4.5-6.5 (skin compatible)
Spreadability (cm)	6.13 ± 0.17	5.60 ± 14	5.04 ± 0.38	5.0-7.0 cm
Adhesiveness (Sec)	5.01 ± 0.20	6.35 ± 0.25	8.21 ± 0.79	> 4 Seconds
Viscosity (cP)	2239±171	2989 ± 141	3579 ± 144	2000 – 4000 cP

Note: Data are presented as mean ± SD from three independent replicates (n=3)

Antibacterial Activity Result of Gamat Leaf Extract Against Test Bacteria

To evaluate the antimicrobial efficacy of the Gamat leaf extract, we conducted a disk diffusion assay against common bacterial pathogens. The resulting measurements of the inhibitory zones, which reflect the extract's ability to suppress bacterial growth at varying concentrations, are summarized in Table 3 below

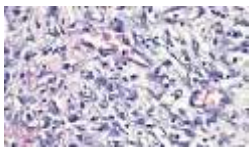
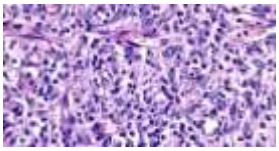
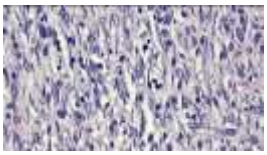
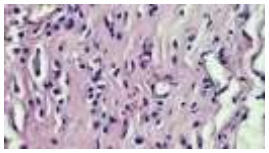
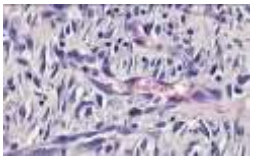
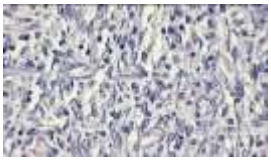
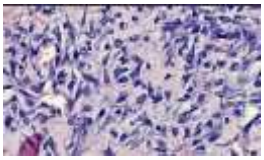
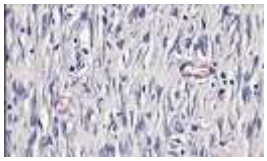
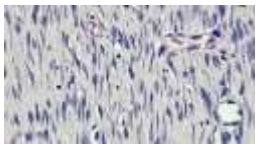
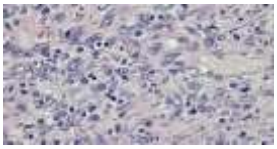
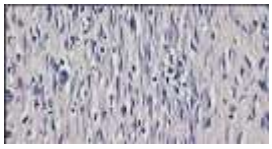
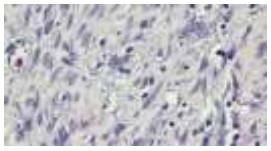
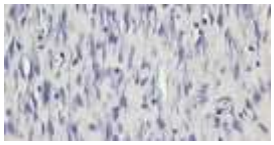
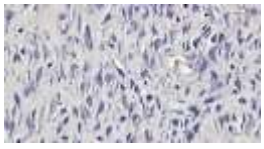
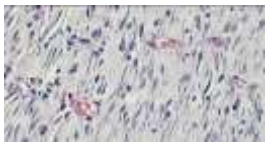
Table 3.
Antibacterial Activity of Gamat Leaf Extract Against Test Bacteria

Extract Concentration (%)	Replicate 1 (mm)	Replicate 2 (mm)	Replicate 3 (mm)	Mean Diameter of Inhibiton Zone (mm)	Activity Category
Control (+)	26	22	23	23.6	Very Strong
Control (-)	0	0	0	0	N/A
5%	0	0	0	0	No Activity
10%	3	2	2	2.3	Weak
15%	2	2	2	2	Weak

The antibacterial screening results, summarized in Table 3, indicate that the Gamat leaf extract's inhibitory effect is concentration dependent up to a certain threshold. While the 5% concentration showed no activity, the 10% group produced a 1.80 mm zone of inhibition. A slight contraction to 1.60 mm was noted at the 15% concentration, a phenomenon likely driven by the extract's increased viscosity. Such physical density may have restricted the radial migration of bioactive metabolites from the disk into the surrounding agar matrix.

Hematoxylin-Eosin (HE) Analysis Results of Rat Wound Tissue

Table 4.
Histopathological Imaging with Hematoxylin-Eosin (HE) Staining in Diabetic Ulcers in *Rattus norvegicus*

Day / To	Positive Control	Gamat Gel Formulation		
		5%	10%	15%
7				
				
14				
				

Hematoxylin-Eosin (HE)-stained ulcer tissue preparations were observed under a microscope at 400x magnification in five fields containing macrophages, fibroblasts, and blood vessels. Interpretation of the mean numbers of macrophages, fibroblasts, and blood vessels is shown in Table 5.

Table 5.
Interpretation of the Mean Numbers of Macrophages, Fibroblasts, and Blood Vessels

Research Variables	Gamat Gel Formulation							
	Positive Control		5 %		10%		15%	
	7 Days	14 Days	7 Days	14 Days	7 Days	14 Days	7 Days	14 Days
Average Macrophage Count	23,3	14,25	24,85	18,05	21,05	13,05	11,7	7,9
Average Fibroblast Count	53,55	71,5	59,2	59,5	65,9	79,95	35,85	86,2
Average Number of Blood Vessels	10,5	6,25	11,75	6,25	15,3	9,45	6,8	17,45

DISCUSSION

Profiling via GC-MS indicate that Gamat leaf extract is characterized by a complex of diterpenes, triterpenes, and various aliphatic derivatives, including aldehydes, ketones, and epoxides. The coexistence of these distinct secondary metabolites points toward a polypharmacological mode of action, which is particularly suited to addressing the staggered healing phases and biochemical complexities inherent in diabetic ulcers. The prominence of triterpenoids and diterpenes in the extract serves as a fundamental pillar for its regenerative properties. In chronic diabetic ulcers, the healing process is often stalled in a persistent inflammatory state. Triterpenes are widely recognized for their ability to suppress pro-inflammatory signaling pathways, such as NF-κB, thereby facilitating the transition from the inflammatory phase to the proliferative phase of repair(Narzary et al., 2023). This chemical evidence correlates with the dense fibroblast proliferation observed in the histological analysis, suggesting that these terpenoids act as molecular triggers for extracellular matrix deposition and collagen maturation (Nagashree et al., 2025).

The identification of aliphatic aldehydes and ketones provides a broader perspective on the extract's protective mechanisms. While the in vitro disk diffusion assay showed only modest antibacterial clearance, these metabolites likely function as critical stabilizers within the local antioxidant microenvironment. By directly scavenging reactive oxygen species (ROS) at the wound interface, these compounds serve as a biological shield for fragile, nascent vessels thereby mitigating the chronic oxidative stress that so often compromises tissue repair in hyperglycemic conditions(Carvalho et al., 2025). The detection of epoxides introduces a compelling facet to the phytochemical makeup of the Gamat extract, notwithstanding their inherent reactivity. When maintained at optimal levels, these natural epoxides are documented to catalyze cellular migration and accelerate re-epithelialization, likely through the targeted modulation of cell-surface receptors (Z. Zhang et al., 2026). It is plausible that the synergy between these highly active species and the stabilizing influence of the aliphatic matrix facilitates efficient wound closure, achieving therapeutic efficacy while circumventing the risks of localized toxicity.

The strategic transition of Gamat leaf extract into a gel-based delivery system was primarily driven by the clinical need to optimize the recovery of chronic diabetic wounds. Unlike liquid tinctures, the semi-solid matrix of a gel offers a superior occlusive barrier, a characteristic that is vital for maintaining a moist wound environment. This physiological state is a well-documented prerequisite for accelerating keratinocyte migration and achieving seamless reepithelialization (Dhivya et al., 2015).From a structural perspective, the gel serves as a sophisticated sustained-release vehicle for the bioactive metabolites previously identified via GC-MS. While the formulation's viscosity appeared to hinder radial diffusion in in vitro agar assays, it provides a distinct clinical advantage by ensuring prolonged contact time at the ulcerated site. This stability allows triterpenoids and diterpenes sufficient time to penetrate the dermal layers, where they can effectively downregulate persistent inflammatory signals and catalyze fibroblast proliferation (Beserra et al., 2019). Furthermore, the hydrophilic nature of the gel base facilitates a controlled release of reactive epoxides and antioxidant ketones. This localized delivery mitigates oxidative stress directly at the wound interface without the rapid evaporation risks associated with ethanol based preparations. The physical integrity and spreadability of the gel further enhance its therapeutic utility. By adhering to

the irregular topography typical of diabetic foot ulcers, the formulation preserves delicate components, nascent vascular structures from mechanical friction and exogenous contaminants. This film forming property, synergized with the phytochemical profile of the Gamat leaf, establishes a microenvironment that mimics the natural extracellular matrix. Such a mechanism effectively bridges the gap between a stalled inflammatory phase and active tissue remodeling (Djumaev & Tashmukhamedova, 2020). Consequently, this optimized topical formulation represents a significant advancement in leveraging local botanical resources for specialized chronic wound care.

Preliminary antibacterial screening of the Gamat leaf extract produced measurable, though relatively modest zones of inhibition against the target pathogens. Data indicate that the 10% and 15% concentrations resulted in clear zones of 1.80 mm and 1.60 mm respectively. Whereas the 5% concentration failed to elicit any inhibitory response. While these dimensions categorize the extract's activity as weak under standard interpretative criteria, the emergence of any discernible inhibition from a raw, crude extract remains scientifically noteworthy especially when compared to the absolute lack of activity in the negative control groups. The antibacterial screening results, summarized in Table 3, indicate that the Gamat leaf extract's inhibitory effect is concentration-dependent up to a certain threshold. While the 5% concentration showed no activity, the 10% group produced a 1.80 mm zone of inhibition. A slight contraction to 1.60 mm was noted at the 15% concentration, a phenomenon likely driven by the extract's increased viscosity. Such physical density may have restricted the radial migration of bioactive metabolites from the disk into the surrounding agar matrix.

An increase in macrophage counts occurred on day 7 after administration of 5%, 10%, and 15% Gamat leaf extract ointment, as well as the positive control. A significant decrease in the average macrophage count occurred with the 10% concentration of Gamat leaf extract ointment on day 14 compared to the 5% and 15% concentrations. On day 14, differences in the average macrophage counts were observed between each treatment. This indicates that Gamat leaf extract extract gel at each concentration was able to reduce the number of tissue macrophages, especially at the 10% concentration. Tissue macrophages play a crucial role in wound healing, particularly during inflammation. Monocytes that enter the tissue during chronic inflammation differentiate into macrophages capable of phagocytosing damaged tissue, including dead polymorphonuclear leukocytes (PMNs). Furthermore, macrophages play a role in granulation tissue formation, along with fibroblasts, angiogenesis, collagen synthesis, and growth factor production during re-epithelialization. When inflammation occurs, tissue macrophages increase drastically (Malaha et al., 2023). Macrophages in wound tissue will help accelerate the wound healing process during the inflammatory process. In the early phase of inflammation, the increasing number of macrophages in the tissue will help accelerate wound healing (Dewi & Setiawan, 2020). Macrophages increase in the inflammatory phase and decrease in the proliferation phase when the wound begins to close (Bonardo, 2015; Dewi & Setiawan, 2021). Increased macrophage activity is characterized by the increase in size and shape of macrophages and the presence of various pseudopods. Tortuous phagosomes, numerous lysosomes, a large Golgi apparatus, and a rough endoplasmic reticulum develop (Bratawidjaja & Regganis, 2014; Ilyas et al., 2020).

In Table 5, the average number of fibroblasts increased on day 14 compared to day 7 in all treatments, this indicates that the wound healing process is assessed by the average number of good fibroblasts in all treatments. The number of fibroblasts present at the wound site is stimulated by Transforming Growth Factor (TGF). The wound healing process is influenced by the proliferation and migration of fibroblasts in the wound area. Fibroblast proliferation in the wound area is a stage of wound healing that occurs rapidly, this process occurs on days 7 to 14 after the wounding process, after which skin refinement occurs until normal (Kanzaki et al, 1998; Wulandari et al, 2023). The number of fibroblasts in the early days of the wound process is not dominant, entering the proliferation phase characterized by fibroblasts present in the wound area, the remodeling phase

on day 7. Fibroblasts will synthesize fibronectrin which is an important component of the formation of the extracellular matrix that plays an important role in the wound healing process (Rahayu, 2022). In Table 5, the increase in fibroblast numbers at a concentration of 15% was the highest compared to other concentrations and the positive control. This indicates that the secondary metabolite compounds in the 15% concentration of Gamat leaf extract can accelerate the process of fibroblast proliferation which is important in the wound healing process. The content of flavonoid secondary metabolites contained in Gamat leaves, especially quercetin, can increase fibroblast migration and anti-inflammatory (Wijaya et al, 2025).

In wound healing, the formation of granulation tissue is characterized by blood vessels or revascularization that can supply substances such as amino acids and glucose to fibroblasts, thereby optimizing collagen formation (Brunicardi et al, 2015; Ningsih et al, 2025). The content of flavonoid compounds can increase the expression of Insulin-Like Growth Factor-1 (IGF-1), which plays a role in collagen synthesis and fibroblast proliferation and inhibits the secretion of neutrophil degradative enzymes (Asri et al, 2021). The highest average number of fibroblasts was found at a concentration of 15%, making this concentration more effective in stimulating the ulcer healing process compared to concentrations of 5 & 10%. The content of metabolite compounds contained in Gamat leaf extract, such as flavonoids and several other compounds, can act as anti-inflammatories and stimulate an increase in fibroblasts (Dewi et al, 2024). The increase in the number of fibroblasts in gamat extract gel is directly proportional to the increase in concentration used. Fibroblasts play an important role in the normal wound healing process. Fibroblasts play a role in the breakdown of fibrin which produces a new extracellular matrix (ECM), collagen synthesis can facilitate wound healing (Paramita et al, 2024). The average increase in blood vessel count at 15% concentration on day 14 showed the highest value compared to the comparison and control concentrations. This indicates that this stage is the proliferative phase of wound healing, characterized by the presence of granulation tissue rich in new blood vessels. This process consists of three main processes, the first of which is neoangiogenesis, which is the natural growth of new blood vessels (Primadina et al., 2019). Flavonoids function by stimulating the production of Vascular Entothelial Growth Factor (VEGF), which plays a role in new blood vessel growth (Wicaksono et al., 2022).

CONCLUSION

This study concludes that Gamat Leaf extract effectively accelerates wound healing. The synergy between bioactive compounds (GC-MS), optimal formula stability (Viscosity), and improved tissue regeneration (HE analysis) proves its potential as a superior and standardized pharmaceutical preparation.

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