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Original Article

Effect of Ethanol Extract of Rosella Flower (*Hibiscus sabdariffa* L.) on the Expression of Fibroblast Growth Factor-2 (FGF-2) and Matrix Metalloproteinase-1 (MMP-1) in the Healing Process of Incision Wounds in White Wistar Rats

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ABSTRACT

Background: This study investigated the effect of rosella flower petal (*Hibiscus sabdariffa* L.) ethanol extract gel on incision wound healing in a rat model of diabetes mellitus. Wounds are skin disorders that can be caused by internal or external factors. The wound healing process involves various growth factors, including Fibroblast Growth Factor-2 (FGF-2) and Matrix Metalloproteinase-1 (MMP-1). **Materials and Methods:** This study used a post-test-only controlled group design with male Wistar rats as the research subjects. This study aimed to evaluate the effect of rosella flower petal ethanol extract gel on FGF-2 and MMP-1 levels, as well as collagen and fibroblast cell formation in the wound healing process. **Results:** Phytochemical screening results revealed the presence of flavonoids, alkaloids, saponins, phenols, and tannins in rosella extract. The results showed a significant difference ($p<0.05$) in FGF-2 levels between all research groups and significant difference ($p<0.05$) in MMP-1 levels. **Conclusion:** The group that received ethanol extract gel of rosella flower petals with a concentration of 12% showed a better increase in the formation of FGF-2, MMP-1, collagen, and fibroblast cells compared to the other groups

Keywords: Ethanol Extract Rosella Flower Petals, Incision Wounds, White rat Wistar strain.

INTRODUCTION

Wounds are disturbances in the normal condition of the skin in the form of damage to its continuity due to pathological processes, both internal and external. Wounds are something that often occurs in everyday life and if not treated adequately can cause serious complications.[1] The incidence of injuries is increasing every year, more than 1.2 million people die globally due to traffic accidents, and around 20-50 million people suffer non-fatal injuries, including injuries. In Indonesia, the number of injuries is quite high in line with the increasing incidence of traffic accidents in the last few decades.[2]

The wound healing process is a complex pathophysiological process because it involves coordinated processes of inflammation and proliferation. Several things are important factors in the wound area, one of which is the Extracellular Matrix (ECM) which is a driver of the wound healing process.[3] The wound healing process is generally divided into several phases, starting with hemostasis, inflammation, proliferation (cellular infiltration, angiogenesis, and re-epithelialization), then the maturation or remodeling phase.[4]

In the inflammatory phase, immune cell activation promotes the secretion of proinflammatory cytokines to influence the migration of fibroblasts, epithelial cells, and endothelial cells. Fibroblasts contribute to collagen deposition. The release of collagen fragment degradation that triggers fibroblast proliferation and growth factor synthesis triggers angiogenesis and re-epithelialization. [5]

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Physiological skin growth factors play a role in wound healing, one of which is Fibroblast Growth Factors (FGF). 1 type of FGF that plays an important role in wound healing is Fibroblast Growth Factors-2 (FGF-2). In skin fibroblasts, FGF-2 plays a role in increasing the synthesis of matrix macromolecules and hyaluronan by stimulating the expression of hyaluronan synthesis genes.[6] FGF-2 contributes to the wound healing process by regulating the synthesis and deposition of various ECM components, increasing keratinocyte motility during re-epithelialization regulating many proteases by upregulating Matrix Metalloproteinases (MMPs), resulting in extracellular matrix remodeling and promoting angiogenesis.[7]

Matrix Metalloproteinase-1 (MMP-1), also known as Collagenase-1, is actively expressed in the wound area during the angiogenesis phase and decreases in the re-epithelialization phase until wound closure occurs.[8] MMP-1 is an enzyme primarily responsible for collagen turnover in most tissues. In a variety of normal and disease-related tissue remodeling events, MMP-1 can be expressed by epithelial cells, fibroblasts, endothelial cells, chondrocytes, and macrophages.[9]

Skin wounds are a serious health problem worldwide and are often associated with high costs and limited treatment efficiency.[10] Wounds that are closed with stitches tend to heal quickly. However, chronic wounds are usually accompanied by infection, inflammation and intense oxidative stress, resulting in suboptimal wound healing,[11] because the pathophysiology of wound healing has extensive factors in biochemical and molecular mechanisms so that the therapeutic strategy is quite challenging.[12]

Hibiscus sabdariffa L. is a shrub belonging to the Malvaceae family that grows in tropical areas such as Java and Kalimantan.[13] Rosella flower's petal are known to have various properties that have been used in the health sector. The potential benefits and uses for treatment are very diverse. *Hibiscus sabdariffa* L. is known to be effective in treating inflammation, oxidative stress, hypertension, hyperlipidemia, carcinogenicity, infections, diabetes, Alzheimer's disease, and many more.[14]

Rosella flower petals are a source of bioactive compounds such as polyphenols (flavonoids, tannins), carotenoids and ascorbic acid. The benefits of rosella flower petals themselves are widely known as antioxidants that fight free radicals. The content of phenolic compounds in roselle flower petal extract shows its activity as an antioxidant such as flavonoids, which are dominated by quercetin, luteolin, gossypetin, and other glycosides.[15] Meta-analysis research conducted by Lucy *et al.*,[16] shows that the bioactive compounds in *Hibiscus sabdariffa* L. have antioxidant and anti-inflammatory effects to reduce the risk of cardiovascular disease.[16] *Hibiscus sabdariffa* L. flower extract exhibits hyaluronic acid-stimulating effects and moderate collagen-stimulating effects without cytotoxicity even at high concentrations.[17] In accordance with the explanation above, the author is interested in conducting research on the effects of ethanol extract gel from rosella flower petals on FGF-2 and MMP-1 on the healing of incised wounds in white rat strains wistar.

MATERIALS AND METHODS

Research Design

This research employed a true experimental design with a posttest-only controlled group design.

Experimental Animals

Male Wistar rats (*Rattus norvegicus*).

Age: 2-3 months.

Weight: 200-250 g.

Housing: Individual cages maintained at 22-24ºC with 12-hr light/dark cycle.

Standard pellet diet and water *ad libitum*.

Materials

Rosella (*Hibiscus sabdariffa* L.) flower petals.

Extraction Materials

Ethanol 96%,

Carbopol 940,

Propylene glycol,

Triethanolamine,

Methylparaben,

Distilled water.

Surgical and Treatment Materials

Surgical blade No. 15,

Sterile surgical gloves,

Povidone-iodine solution,

Local anesthetic: Lidocaine 2%,

Sterile gauze,

Cotton swabs.

Equipment

Laboratory Equipment

Rotary evaporator,

Digital analytical balance,

pH meter,

Homogenizer,

Centrifuge.

Surgical Equipment

Surgical scissors,

Forceps,

Digital caliper for wound measurement,

Surgical lamp.

Sample Analysis Equipment

Microscope,

Tissue processor,

Microtome.

Study groups

Research was carried out at the Universitas Methodist Indonesia Medical Phytochemistry Laboratory to make ethanol extract gel of roselle flower petals and phytochemical screening of roselle flower petals, examination of flavonoids and phenolics. The animal laboratory begins the acclimatization process, providing treatment for incision wounds and providing surgical treatment for experimental animals. UMI Faculty of Medicine Integrated Laboratory for examining FGF-2, MMP-2 levels and extensive histopathology of wounds.

The experimental animals used in this research were male white Wistar rats. The selection of mice as experimental animals was based on the consideration that genetically, mice are similar to humans and have the ability to adapt to the laboratory environment. Sample allocation (grouping) using inclusion and exclusion criteria. The inclusion criteria in this study were mice aged 2.5-3 months, body weight 150-200 g, male gender, incisions had been made and healthy condition (active and not disabled). The exclusion criteria in this study were that male white rats died during the study period.

Each treatment group contained a minimum of 5 male mice. Researchers chose to use 6 male rats per group to maintain the mortality of experimental animals with a total of 5 treatment groups so that the total number of research samples was 30, which were divided into:

a. Group 1 (K1), negative control, was given base/placebo gel.

b. Group 2 (K2), Bioplacenton® Positive control.

c. Group 3 (K3), was given roselle flower petal extract gel with 6%.[18]

d. Group 4 (K4), was given roselle flower petal extract gel with 12%.

e. Group 5 (K5), was given roselle flower petal extract gel with 15%.

Making ethanol extract of rosella flower

Plant Material Source

Rosella (*Hibiscus sabdariffa* L.) flowers were collected from North Sumatera, Indonesia". Ethanol extract of rosella flowers was obtained by maceration method using 96% alcohol solvent with a ratio of 1: 5. The simplicia and solvent were soaked in a vessel for 4 days. The filtrate and dregs were separated with a buchner equipped with a vacuum. The dregs were remacerated 2 times. The filtrate was concentrated with a rotary evaporator and water bath.

Making ethanol extract gel of rosella flower

Carbomer (*Polyacrylic acid*) weighing 2 g per formulation was dispersed in distilled water (10 times the amount of carbomer) stirred, triethanolamine was added and then stirred slowly until a gel mass was formed. Carbomer functions as a gel base that will carry the active substance from the rosella extract and provide good consistency to the preparation. The results of the extraction of rosella flowers are mixed with glycerin and nipagin then the propylene glycol is dissolved in distilled water. The mixture obtained was added to the gel mass and then stirred until homogeneous. Formulation of roselle flower petal extract gel preparations into three different concentrations (6%, 12%, and 15%).[18-20]

FGF-2 measurement

FGF-2 levels were measured using the ELISA (Enzyme linked Immunosorbent Assay) method. Specimens were taken from blood plasma on the 21st day. Procedures for taking and storing samples, preparing reagents, examining assays and calculating results follow the rules set by the Bioassay Technology Laboratory company.[21]

MMP-1 Measurement

MMP-1 levels were measured using the ELISA (Enzyme Linked Immunosorbent Assay) method. Specimens were taken from blood plasma on the 21st day. Procedures for taking and storing samples, preparing reagents, administering assays and calculating results follow the rules set by the Bioassay Technology Laboratory company.[22]

Histological Examination Using Hematoxylin Eosin

Histopathological observations were carried out on skin samples taken on the 21-day post wounding. The observations made were histopathological observations by scoring each healing parameter (re-epithelialization, formation of connective tissue and collagen as well as the number of inflammatory lymphocyte cells).[23]

Statistical analysis

Data obtained from observations were recorded and presented in the form of mean ± standard deviation. Data normality and

homogeneity tests were carried out. If the data is normally distributed and homogeneous then an ANOVA test is carried out. All data analysis was carried out using SPSS software. In this study, for statistical test decisions, a real level of 5% (p=0.05) was taken which was considered meaningful or significant.

Ethical statement

The use and handling of experimental animals in research laboratories is carried out in accordance with the ethical rules for animal research regulated in the Declaration of Helsinki and obtained ethical clearance from the ethics committee of the Faculty of Medicine, Universitas Methodist Indonesia No. 06/KEPK-FKUMI/EC/2024, Medan.

RESULTS

This study constitutes a laboratory-based experimental investigation employing a post-test only controlled group research design. It was conducted at the Phytopharmaceutical Laboratory, Veterinary Laboratory, and Integrated Laboratory of the Faculty of Medicine, Universitas Methodist Indonesia.

Determination Analysis of *Hibiscus sabdariffa* L.

Determination of sample materials in this study using (Table 1).

Based on the results of our identification and determination, the sample sent was Rosella (*Hibiscus sabdariffa* L. var. *sabdariffa*).

Classification

Kingdom: *Plantae*

Division: *Magnoliophyta*

Class: *Magnoliopsida*

Order: *Malvales*

Family: *Malvaceae*

Genus: *Hibiscus*

Species: *Hibiscus sabdariffa* L.

Phytochemical Screening Analysis of Ethanol Extract of *Hibiscus sabdariffa* L.

Phytochemical Screening Results of *Hibiscus sabdariffa* L. Petals (Table 2).

Bivariate Analysis

Analysis of the Relationship between Differences in Activity of Serum FGF-2 Levels between Groups of White Wistar Rat with Incision Wounds.

Results of analysis of the relationship between differences in serum FGF-2 levels between groups of male white rats with Incision Wounds after administration of *Hibiscus sabdariffa* L. ethanol extract gel (Table 3).

In this study, 30 samples were divided into 5 groups. Group 1 had samples with FGF2 levels of Mean=320.61 and SD=94.43, group 2 Mean=366.66 and SD=60.12, group 3 Mean=260.26 and SD=47.11, group 4 of Mean=460.11 and SD=134.09 and group 5 Mean=313.24 and SD=74.60. The results of this analysis showed a significant relationship between groups (p<0.001).

Analysis of the Relationship between Differences in the Activity of Serum MMP-1 Levels Between Groups of White Wistar Rat with Incision Wounds

Results of analysis of the relationship between differences in serum MMP-1 levels between groups of male white rats with Incision Wounds after administration of *Hibiscus sabdariffa* L. ethanol extract gel (Table 4).

In this study, 30 samples were divided into 5 groups. The group 1 obtained samples with MMP-1 levels of Mean=2.66 and SD=0.40, the group 2 had Mean=2.68 and SD=0.49, the group 3 had Mean=2.36 and SD=0.56, group 4 Mean=2.75 and SD=0.78 and group 5 Mean=3.69 and SD=0.77.

Histopathological examination of wounds with incisions in male Wistar white rats following the administration of *Hibiscus sabdariffa* L. ethanol extract gel.

Results of histopathological analysis of wounds from male white rats with incision wounds after administration of *Hibiscus sabdariffa* L. ethanol extract gel (Table 5).

Wound histopathology results in this study from 30 samples showed that the highest average collagen density was in the group 4 compared to other groups. The highest number of fibroblast cell formations were in group 3 compared to other groups. Meanwhile, the lowest inflammation score was in group 4 compared to the other groups.

The histopathological description of the extent of the wound, collagen density, fibroblast cells and inflammation is shown in Figures 1-3.

DISCUSSION

Wounds are a health problem that we often encounter. A wound is a loss of tissue continuity, can occur anywhere and at any time, and may not be recognized as dangerous.[24] The principle of wound care is to achieve rapid wound closure, functional and aesthetic scar restoration.[25,26] This goal is achieved through processes that prevent infection and further trauma, as well as providing an optimal environment for the wound healing process itself.[25,27]

Table 1: Determination of *Hibiscus sabdariffa* L.

Name	Kind	Tribe
Rosella flower	*Hibiscus sabdariffa* L. var. *sabdariffa*	Malvaceae

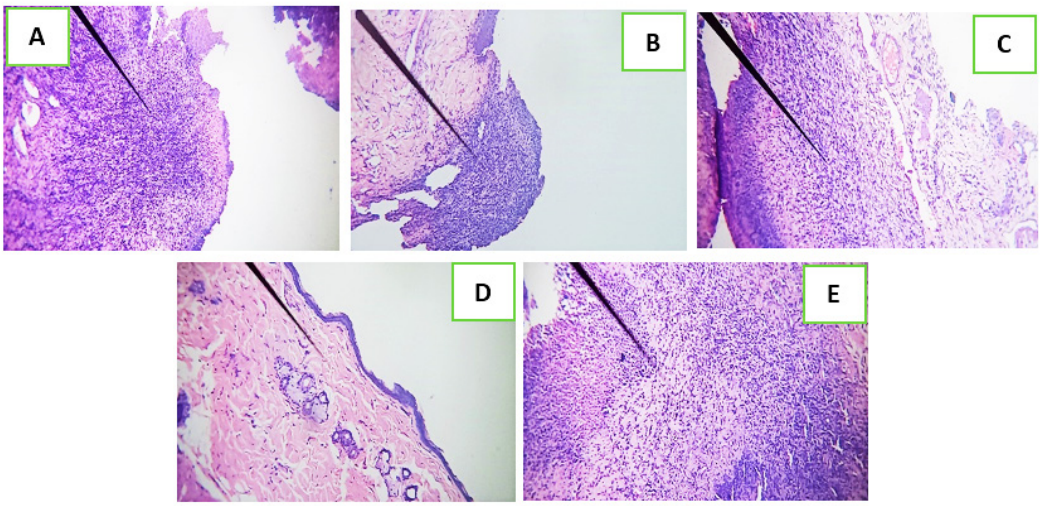

Figure 1: Histopathological features of collagen density in wound healing (H and E staining, 400× magnification, scale bar=50 µm). (A). Group 1 collagen density; (B). Collagen density group 2; (C). Collagen density group 3; (D). Collagen density group 4; (E). Group 5 collagen density.

Table 2: Phytochemical Screening of Ethanol Extract of *Hibiscus sabdariffa* L.

Sl. No.	Component	Picture	Results
1	Flavonoid		(+)
2	Alkaloids (bouchardad mayer dragendrof)		(+)
3	Saponins		(+)
4	Tannin		(+)
5	Phenol		(+)

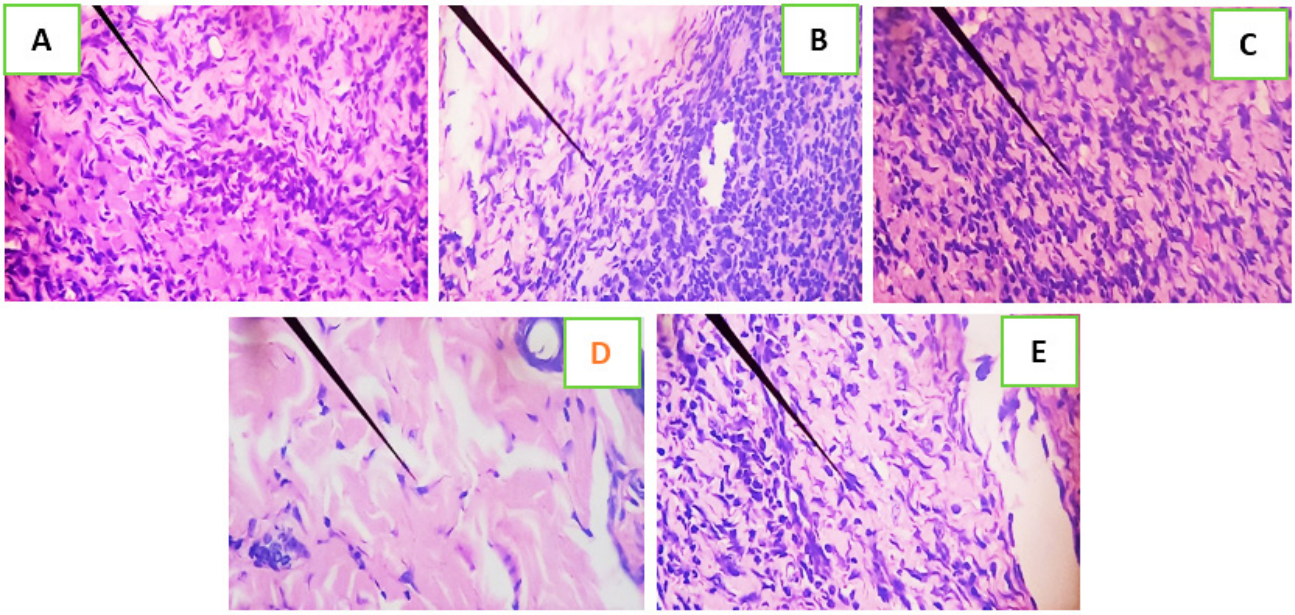

Figure 2: Histopathological features of fibroblast cells in wound healing (H and E staining, 400×magnification, scale bar=50 µm). (A). Group 1 fibroblast cells; (B). Group 2 fibroblast cells; (C). Group 3 fibroblast cells; (D). Group 4 fibroblast cells; (E). Group 5 Fibroblast cells.

Table 3: Results of Relationship between Differences in Activity of Serum FGF-2 Levels between Groups of White Wistar Rat with Incision Wounds.

Group	Mean±SD	*p*
K1	320.61±94.43	
K2	366.66±60.12	
K3	260.26±47.11	0.008*
K4	460.11±134.09	
K5	313.24±74.60	

*Anova Test (Significant <0,05).

Ethanol extract of Rosella flower is formulated in gel dosage form. Researchers chose this gel formulation because of the cool sensation on the skin, good spreadability, minimal scarring, clear appearance, and good release of active ingredients. Gels are semisolid formulations consisting of small or large molecules in a liquid solvent and prepared by adding a gelling agent, such as jelly.[28] The gel is easy to apply and spread, making it easy and quick to apply to the skin. In addition, the gel is cooling, moisturizing, and easily absorbed into the skin, providing a healing effect on wounds.[29]

Phytochemical Screening Results of Ethanol Extract of *Hibiscus sabdariffa* L.

This research carried out a phytochemical screening examination of the ethanol extract of *Hibiscus sabdariffa* L., secondary metabolites were obtained such as: flavonoids, alkaloids and tannins (Table 2), this means that rosella flower petals have great potential as antioxidants. This is the same as research by Shafirany *et al.*,[30] that purple rosella flowers (*Hibiscus sabdariffa* L.) have very strong antioxidant activity because they contain flavonoids, alkaloids, tannins and saponins which can ward off free radicals.[30] Research by Obouayeba *et al.*,[31] that the ethanol extract of rosella petal contains alkaloids, flavonoids, saponins and tannins. *Hibiscus sabdariffa* L. is a potential source of natural antioxidants, and this justify its uses in folkloric medicines.

Results of Analysis of Differences Between and Within Activity Groups in FGF-2 Levels of Male Wistar White Rats Incision Wounds After Administration of Ethanol Extract of Rosella Flower Petal.

The results of this study showed a significant relationship between groups (p=0.008) (Table 3). These results show that ethanol extract of *Hibiscus sabdariffa* L.can increase FGF-2 levels in the blood of male white Wistar rats using an incised wound model. Rosella flower petal extract has a high antioxidant content derived from flavonoids, Gossypentin, Quarcetin, Antocyanin.[32] Quarcetin is 1 of the active substances in the flavonoid group from the flavonol group which has very high free radical activity. Antioxidants from the Quarcetin compound are able to trigger collagen production and increase Vascular Endothelial Growth Factor (VEGF).[33] Previous research (Narmada *et al.*, 2021) found that anthocyanin content can reduce the secretion of pro-inflammatory cytokines while increasing VEGF and FGF-2, both of which are markers of neovascularization.[34] The saponin compound contained in the ethanol extract of *Hibiscus sabdariffa* L. can increase monocyte proliferation which can increase the number of macrophages. Macrophages will produce growth factors such as Platelet-Derived Growth Factor (PDGF), Fibroblast Growth Factor (FGF), Epidermal Growth Factor (EGF), and Transforming Growth Factor-β (TGF-β).[35]

Results of Analysis of Differences Between Activity Groups in MMP-1 Levels of Male Wistar White Rats Incision Wounds After Administration of Ethanol Extract of *Hibiscus sabdariffa* L.

The results of this study showed a significant relationship between groups (p=0.019) (Table 4). These results show that the ethanol extract of rosella flower petals can increase the blood levels of MMP-1 in the blood of male white Wistar rats using an incised wound model. The flavonoids in rosella flower petals act as antioxidants which can reduce the level of free radicals in cells and have the ability to increase the concentration of the extracellular matrix through inhibiting MMPs activity. Extracellular matrix deposition will then speed up wound healing time by increasing the strength of the tissue around the wound.[36,37]

Table 4: Results of Relationship between Differences in Activity of Serum MMP-1 Levels Between Groups of White Wistar Rat with Incision Wounds.

Group	Mean±SD	*p*
K1	2.66±0.40	
K2	2.68±0.49	
K3	2.36±0.56	0.019*
K4	2.75±0.78	
K5	3.69±0.77	

*Anova Test (Significant <0.05).

Flavonoids have the effect of accelerating the wound healing process due to their antimicrobial and astringent activity which is responsible for the process of wound contraction and wound epithelialization. This epithelialization process only stops after the epithelium touches each other and covers the entire wound surface.[38,39] Flavonoids have the ability to act as antioxidants which can reduce free radicals. Flavonoids can also function as destroyers of microbes, especially gram-negative bacteria. Flavonoids have an anti-bacterial mechanism by forming complex compounds with soluble and extracellular proteins that can damage bacterial cell walls, followed by the release of intracellular compounds.[40] Tannins and alkaloids have antioxidant and antimicrobial properties which help the wound healing process by preventing and protecting the wound area from damage caused by free radicals and inhibiting the growth of pathogenic bacteria in wounds.[41]

Results of Histopathological Analysis of Wounds of Male Wistar White Rats Incision Wounds After Administration of Ethanol Extract Gel of *Hibiscus sabdariffa* L.

Table 5: Results of Histopathological Analysis of Wounds of Male Wistar White Rats Incision Wounds After Administration of *Hibiscus sabdariffa* L. ethanol extract gel.

Group	Collagen Density (x̄)	Fibroblast Cells (x̄)	Inflammation (x̄)
K1	0.33	24.67	3
K2	0.33	38.67	2.33
K3	0.67	76.67	3
K4	4.00	5.33	0
K5	0	67.33	3

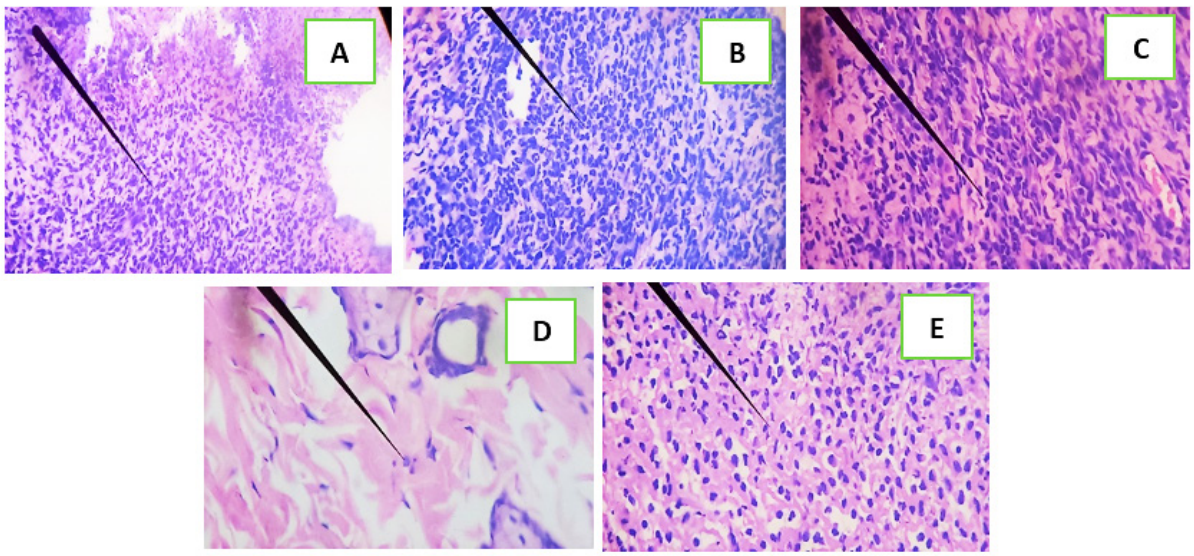

Figure 3: Histopathological features of inflammatory cell infiltration in wound healing (H and E staining, 400×magnification, scale bar=50 µm). (A). Inflammatory Cells group 1; (B). Group 2 inflammatory cells; (C). Group 3 inflammatory cells; (D). Group 4 inflammatory cells; (E). Group 5 inflammatory cells.

The findings from histopathological investigation of wounds in rats using the incisional wound model indicated that group 4 exhibited superior progress in the wound healing process. This superiority was evident in the enhanced formation of collagen and a reduction in inflammatory cells, as depicted in Table 5. Additionally, when considering the formation of fibroblast cells, group 5 surpassed the other treatment groups, as shown in Table 5.

Several growth factors, including Transforming Growth Factor β1 (TGF-β1) and FGF, regulate the increase in collagen synthesis. Flavonoids play a role in accelerating the formation of FGF, thereby expediting the synthesis of new skin tissue to facilitate wound closure.[42]

Additionally, vitamin C aids in the wound healing process by promoting increased collagen synthesis. Collagen, a protein substance known for enhancing the surface tension of wounds, can strengthen tissue within the wound by increasing its density.[43]

The flavonoids present in the ethanol extract gel of rosella flower petals act as antioxidants, reducing free radicals. They also possess antimicrobial properties, particularly against gram-negative bacteria. Flavonoids function as antibacterial agents by forming complex compounds with soluble and extracellular proteins, which can damage bacterial cell walls and release intracellular compounds.[44] Tannins and alkaloids, on the other hand, possess antioxidant and antimicrobial properties that contribute to the wound healing process. They help prevent and protect the wound area from damage caused by free radicals and inhibit the growth of pathogenic bacteria in wounds.[45]

CONCLUSION

The results of the phytochemical screening of the ethanol extract of rosella flower petal are flavonoids, alkaloids, saponins, tannins and phenols. The application of 12% rosella flower petal ethanol extract gel demonstrates superior efficacy in promoting the formation of FGF2, MMP-1, collagen, and fibroblast cells during the healing process of incisional wounds

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

FGF-2: Fibroblast Growth Factor-2; **MMP-1:** Matrix Metalloproteinase-1; **ECM:** extracellular matrix; **MMPs:** Matrix metalloproteinases; **ELISA:** Enzyme linked Immunosorbent Assay; **VEGF:** Vascular Endothelial Growth Factor; **TGF-β1:** Transforming Growth Factor.

AUTHOR CONTRIBUTION

The authors have made substantial contributions to conception, literature survey and drafting the review.

ETHICAL APPROVAL

All experimental procedures in this study were carried out in accordance with the Institutional Animal Care guidelines of the Animal House of the Faculty of Medicine, Methodist University of Indonesia and approved by the Ethics Committee of the Faculty of Medicine, Methodist University of Indonesia (code of ethics: No. 6/KEPK-FKUMI/EC/2024).

SUMMARY

This study explored the efficacy of *Hibiscus sabdariffa* L. (Roselle) flower petal ethanol extract on wound healing in diabetic Wistar rats. Using a post-test only controlled group design, researchers investigated the extract's impact on Fibroblast Growth Factor-2 (FGF-2) and Matrix Metalloproteinase-1 (MMP-1) expression, key factors in the wound healing process.

Phytochemical screening revealed the presence of flavonoids, alkaloids, saponins, tannins, and phenols in the roselle extract. The study demonstrated significant differences in FGF-2 and MMP-1 levels among treatment groups. Notably, the 12% roselle extract gel showed superior results in enhancing FGF-2, MMP-1, collagen formation, and fibroblast cell development.

Histopathological examinations corroborated these findings, with the 12% extract group exhibiting higher collagen density and lower inflammation scores, indicating improved wound healing. These results suggest that roselle flower petal ethanol extract, particularly at a 12% concentration, has potential therapeutic benefits in accelerating incision wound healing in diabetic conditions.

This research provides valuable insights into natural product applications in wound management, opening avenues for further investigation and potential clinical applications in treating diabetic wounds.

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