The Effect of the **Mahkota Dewa** (*Phaleria macrocarpa*) on the Density of Collagen Incision Wounds

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**Abstract.** Wounds are a disorder in the continuity of the skin or mucosal epithelial lining which is a result of physical or thermal damage [1]. Wound healing is an interesting mechanism and an important step to complete the wound closure [2]. There are three phases in the wound healing process. These phases are the inflammatory, proliferative and maturation phases [3]. The stages of wound healing in each phase are influenced by many factors [4]. Early treatment and proper wound care are essential to accelerate the wound healing process [5].

The use of herbal medicines from plants is now increasingly in demand by the community as an alternative therapy that is no less important when compared with medical therapy and has mild side effects. The content of natural ingredients is generally balanced and neutralizing each other [6]. One type of medicinal plant used as an herbal medicine in Indonesia is *mahkota dewa* (*Phaleria macrocarpa*). *Mahkota dewa* (*Phaleria macrocarpa*) mainly distributed in Southeast Asia, especially in Indonesia, which has the effect on treating numerous types of diseases such as hypertension, diabetes, cancer, dysentery, rheumatism, kidney disorder, antihistamines, anti oksidans and antitumor [7]. This plant produces anti-inflammatory activity so that it will reduce the level of inflammation [8] [9]. The active substances contained carbohydrates, saponins, tannins, alkaloids, flavonoids, polyphenolic glycosides, palmitic acid, phenols, terpenoids, doxocanoic acid, lignans,ethyl stearate, and polyphenolic compounds [10]. Saponins have high benefits. Saponins activate and enhance TGF-b1 synthesis, while modifying TGF-b receptors in fibroblasts, and saponins facilitate the TGF-b pathway in wound healing mechanisms [11]. Saponins also enhance the synthesis of pro-collagen [12]. Because of the high potential of the *Mahkota dewa*, it will be very beneficial to the community if conducted research on the effect of the Mahkota dewa on the density of collagen.

**Key word:** Collagen Density, Incision Wound, *Mahkota Dewa* Extract, White Rats

1. **Introduction**

Wounds are a disorder in the continuity of the skin or mucosal epithelial lining which is a result of physical or thermal damage [1]. Wound healing is an interesting mechanism and an important step to complete the wound closure [2]. There are three phases in the wound healing process. These phases are the inflammatory, proliferative and maturation phases [3]. The stages of wound healing in each phase are influenced by many factors [4]. Early treatment and proper wound care are essential to accelerate the wound healing process [5].

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2. **Literature Review**

Wound is defined as the severity of epithelial tissue integrity [13]. Wound healing is a complex process that is very important in maintaining skin function as the body's first barrier [14]. The phases of wound
healing process consists of inflammatory, proliferative, and maturation/remodeling phases. The inflammatory process is one of the wound healing phases which is characterized by the presence of leukocytes (neutrophil activity and macrophages). The proliferative phase is responsible for closure of the lesion, which includes angiogenesis, fibroplasia and reepithelialization. The main purpose of the maturation phase is to achieve maximum tensile strength through reorganization, degradation, and resintesis of the extracellular matrix [15].

3. Research Method
Research sample in this study was 30 male white rats (*Rattus norvegicus*). The sampling technique in this study used simple random sampling. The research design used post-test only control group design. The variables are independent and dependent variable. The independent variable in this study was *Mahkota Dewa* (*Phaleria macrocarpa*) extract. The dependent variable in this study is collagen density. The data collection is carried out in the following way: white rats were randomly selected and divided into control group and treatment group. The control group was given 1% CMC solvent orally. The treatment group was given mahkota dewa extract orally with a dose of 1 mg/Kg bw. The control group was divided into three groups, including KK 1, KK 2, and KK 3 group. The KK 1 group was observed on the first day, then the KK 2 group which network was observed on the fifth day, and the KK 3 group which network was observed on the tenth day. The treatment group was given mahkota dewa extract orally with a dose of 1 mg/Kg bw. The treatment group was also divided into three groups, including KP 1, KP 2, and KP 3 group. The KP 1 group which tissue was observed on the first day, the KP 2 group which tissue was observed on the fifth day, and the KP 3 group which tissue was observed on the tenth day. Observation of wound tissue was made by cutting the wound tissue including healthy tissue about 0.5 cm from the wound and include the muscle under the wound. This observation was taken on day 1, 5, and 10. The results of this observation was wound tissue samples The wound tissue sample was placed and wrapped using filter paper which was given a hole and then fixed by inserting it into 10% formalin for 4-5 days. The data collection was finished by looking at the histological preparations of wound tissue under a light microscope. Wounds were evaluated from day 1st, 5th, and 10th.

4. Result and Discussion
Descriptive analysis of collagen could be seen in Figure 1

![Figure 1. Graph of average collagen density](image_url)

The Figure 1 described the average collagen density in each group. Based on Figure 1, the results showed that the average collagen density in the two groups was still low even though the treatment group had a higher mean than the control group on the first day. On the fifth day, the collagen density in the both groups was increased. On the tenth day, the collagen density in the both groups was also increased, but the treatment group was increased significantly. Based on the Kruskall Wallis test the p-value was 0.001. The p-value was less than α (0.05). So that, there were a significant difference between control and the treatment groups.
Table 1. Mann-Whitney Test Results

| Variable | Group | P value |
|----------|-------|---------|
| Collagen | K1 P1 | 0.011*  |
|          | K2 P2 | 0.56    |
|          | K3 P3 | 0.09*   |

The results of the analysis in table 1 showed that there were significant differences between the control group 1 (K1) and the treatment group 1 (P1) and between the control group 3 (K3) and the treatment group 3 (P3).

The wound healing is a complex and dynamic process that replacing damaged cellular structures and tissue layers [16]. Cellular and biochemical events in wound repair could be divided into several phases as follows: the inflammatory phase, the cell proliferation phase, synthesis of the elements that form the extracellular matrix, and the posterior period called the remodeling or maturation phase [17].

Incisional injury is a wound caused by a sharp instrument [18]. When the injury occurred, the skin would be damaged. The cell membrane damage would eventually stimulate the metabolism of arachidonic acid. In the cyclooxygenase pathway, arachidonic acid metabolism causes the release of inflammation mediators, namely prostaglandins [19]. Prostaglandins cause inflammatory processes [20].

The inflammatory process is one of the wound healing phases, which is characterized by the presence of leukocytes (neutrophil activity and macrophages) [15]. Neutrophils first, followed for the next 8 to 12 hours by slow-moving monocytes. Monocytes then grow and mature into macrophages in the next 8 to 12 hours [21].

Neutrophils and macrophages will produce a free radical called Reactive Oxigen Species (ROS). ROS is a part of the immune system that functions to cleanse wounds from bacteria. Also, ROS can be a signal to modulate various signaling pathways to regulate blood coagulation, thrombosis, migration, proliferation, fibrosis and angiogenesis at each stage of wound healing. ROS molecules appear in the low hydrogen peroxide level. However, in high levels, ROS can damage the tissue heavily. Thus, the presence of ROS in the body for a long time can inhibit the healing process of wounds [22].

*Mahkota Dewa* extract has an active substance that is able to detoxify ROS, namely flavonoids [23]. Flavonoids are powerful antioxidants that can protect the body from ROS. Thus, the wound healing process goes well [24].

Flavonoid compounds also play a role in activating macrophages [25]. This increasing of macrophages will cause an increasing in the secretion of Transforming Growth Factor - β (TGF-β) because TGF-β is produced by all cells, especially platelets, neutrophils, and macrophages (monocytes) [26]. TGF-β serves to trigger proliferation, migration, fibroblast proliferation, storage of extracellular matrix, and stimulus of endothelial cells that function to form new blood vessels. The extracellular matrix is a type 3 collagen type which will later be replaced with type 1 collagen during the remodeling phase. Therefore, the increasing of TGF-β could increase the fibroblast proliferation which will eventually increase fibroblasts [27].

Saponins also have an important role in the wound healing process which is to stimulate fibronectin synthesis by fibroblasts and change the expression of TGF-β receptors [11]. Fibronectin is a large glycoprotein that has an area that binds to several macromolecules such as collagen, proteoglycans, fibrin and heparin. Fibronectin can also bind to cells through the integrin receptor [28]. Fibronectin is found early in the wound healing phase and is able to induce fibroblast migration. When fibronectin is stimulated by fibroblasts, fibronectin migration is fast as well. The fibroblasts will be used for the next wound healing phase to produce collagen [29]. The higher fibroblasts number that move to the wound, the higher collagen synthesized by fibroblasts. The more number of fibroblasts, the more collagen will be produced as well. Collagen will be stored in the extracellular matrix. As well known that the extracellular matrix is a large pile of collagen [30]. With the presence of new collagen produced by fibroblasts, old and new collagen overlap and cause collagen in the extracellular matrix to become thicker, causing the wound to heal faster.

5. Conclusions
Based on the results of this study, the *Mahkota dewa* (*Phaleria macrocarpa*) extract could increase the density of collagen in white rats (*Rattus norvegicus*) with incision wounds.
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