

Wound Healing Activity of Aucubin on Hyperglycemic Rat

Kartini*, Ridho Islamie, Claudia Stefanie Handoyo

Faculty of Pharmacy, University of Surabaya, Surabaya, INDONESIA.

ABSTRACT

Objective: Impaired wound healing in diabetic patients is a serious complication leading to amputation and even death. Proper diabetic wound management is needed to improve the quality of life of diabetic patients. *Plantago major* (plantain) has been used empirically for wound healing. One of its chemical compounds, aucubin, has been studied on non hyperglycemic wound. This study was conducted to determine the wound healing activity of aucubin on hyperglycemic rats, as a model for diabetic wound. **Methods:** A total of 20 hyperglycemic male rats (Wistar) were divided into 4 groups (P1, P2, K1, and K2), and subsequently treated with gel of aucubin 20 µg and 40 µg, bioplacenton®, and gel base, respectively. The fifth group, P3, was normal rats treated with gel of aucubin 40 µg. Drugs were applied topically on animals' wounds induced on the dorsal part (length of 2 cm, a depth of 5 mm), once daily during 21 days. Wound healing activity was evaluated based on the percentage of wound closure

and wound healing time. **Results:** The results showed that the gel of aucubin at the dose of 20 µg and 40 µg could increase the percentage of wound closure (100%) compare to the negative control (83%). Moreover, gels of aucubin accelerated wound healing time (11.7 days) compared to the negative control (24.4 days). **Conclusion:** Aucubin can be used as a drug candidate for diabetic wound healing.

Key words: Aucubin, Diabetic Wound, Hyperglycemic, Wound Healing.

Correspondence

Kartini, Faculty of Pharmacy, University of Surabaya, Surabaya, INDONESIA.

Phone: +62 31 2981110

Email: kartini@staff.ubaya.ac.id

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INTRODUCTION

Aucubin (Figure 1) is an iridoid glycosides widely distributed in plants such as *Aucuba japonica*, *Euphrasia officinalis*, *Eucommia ulmoides*, and so on.¹ Kartini *et al.* isolated and evaluated the activity of this compound from *Plantago major*.² Aucubin activities have been extensively studied, such as for anticancer,²⁻³ immunomodulator,² and for the treatment of mucous membrane inflammation of nasopharyngeal channels.⁴ In addition, aucubin also has wound healing activity on oral mucous and dermal wound,⁵ as well as inhibit the growth of *Escherichia coli* and *Staphylococcus aureus*.⁶ However, so far there has been no report on aucubin activity for diabetic wound healing.

Diabetic wound differs from wound under normal conditions. High blood sugar is a good medium for bacterial growth, and therefore in diabetic ulcer patients most likely will have an infection. In addition, hyperglycemic patients usually experience ischemia and this condition inhibits the wound healing process. Around 6.2% of Indonesian population (20-79 years) suffer from diabetes, 8.7% among of them undergo diabetic foot ulcers (DFU) and 1.3% to be amputated.⁷ Diabetic foot ulcer is one

of the most common chronic complications marked by injury and inflammation in the area under the ankle. This wound should be treated immediately in order to avoid damage leading to amputation. Wound management usually performed for DFU are debridement, maintaining wound moisture, controlling inflammation and infection, and epithelial edge advancement. One of the most common attempts is to control inflammation and infection by using topical antiinflammatory and antibacterial.⁸

It is therefore necessary to perform a further research to evaluate the effects of aucubin on the wound healing process under hyperglycemic conditions. This study was conducted on hyperglycemic male Wistar rat. Aucubin was administered topically on the dorsal portion of the injured rat, then the percentage of wound closure and the closing time of the wound were observed.

MATERIALS AND METHODS

Materials

Chemicals used in this study were aucubin (Sigma, St. Louis, MO, USA), alloxan monohydrate (Sigma, St. Louis, MO, USA), normal saline (Otsuka, Indonesia), Ketalar® (Pfizer), Bioplacenton® (Kalbe Farma, Indonesia), CMC-Na, glycerine, propylene glycol, demineralised water, alcohol, and hair removal cream (Veet®).

Animals

Male Wistar rats (100-200 g) were used in this research. Animals were housed under the conditions of room temperature, 70% of relative humidity, and a 12-h light-dark cycle. They were supplied with standard diet and water ad libitum and acclimatized for two weeks before the experiment. All animals were carried out according to the institutional rules concerning animal experiments (approval No: 721-KE; Dated 15 June 2017).

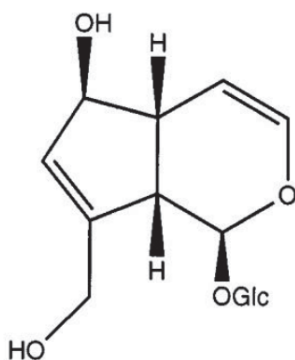


Figure 1: Chemical structure of aucubin.

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Table 1: Composition of Aucubin Gel.

Ingredient	Composition (%)	
	P1	P2
Aucubin	0.04	0.08
CMC-Na	4	4
Glycerin	8	8
Propylene glycol	4	4
Demineralsed water up to	100	100

Preparation of aucubin gel

The dose of aucubin used in this study referred to previous study. Shim *et al.* concluded that the effective dose of aucubin was 50 µl of 0.04% and 0.08% solution which were equivalent to 20 µg and 40 µg aucubin, respectively.⁹ In this recent study, aucubin was formulated into gel dosage form and administered in 1 FTU (Finger Tip Unit, 0.5 g of gel). One FTU of gel contained 20 µg and 40 µg of aucubin, respectively. Composition of aucubin gel is presented on Table 1.

Induction of hyperglycemic

Animals were weighed and their fasting blood glucose levels were determined using Autocheck[®] (Germany) before induction. Animals showing very low or high glucose levels were replaced. The animals were then injected with a single dose of alloxan monohydrate (160 mg/kg BW) in normal saline by i.p. route. Control animals (non-hyperglycemic rats) were injected with normal saline. Fasting blood glucose level was measured two days later to confirm their hyperglycemic status. For blood glucose measurement, the blood was drawn from tail vein. Animals are considered hyperglycemic if their random blood glucose level reached ≥ 200 mg/dL.

Incision wound model

Animals were anaesthetized with ketamine HCl (Ketalar[®], 100 mg/kg BW, i.p.). Incision wound was induced on the dorsal surface with length of ± 2 cm and depth of ± 5 mm using a scalpel. The entire wound was left open. Animals were closely observed for any infection and those which showed signs of infection were separated, excluded from the study and replaced. Animals were then divided into five groups with 5 rats per group, as shown on Table 2. All treatments were carried out topically once daily by application of 1 FTU (Finger Tip Unit, 0.5 g) of gels on the wound. Wound lengths were measured on days of 0, 5, 10, and 21 using digital caliper.

Statistical analysis

Wound healing was evaluated using parameters of the percentage and time of wound closure. The length of the wound was measured using digital caliper on the day 0, 5, 10, and 21. The percentage of wound closure was determined by using the formula: $((L1 - L2) / L1) \times 100$, where L1 is the length of wound on the day 0, while L2 is the length of wound on the day of observation. Time of healing is the day when the wound closes perfectly. All data were then analyzed by one way ANOVA using GraphPad Prism statistical software (GraphPad Software Inc., San Diego, California, Windows Version 5.01). Differences were considered statistically at $P < 0.05$.

RESULTS

Effect of aucubin on the wound closure

Effect of aucubin on the wound closure of hyperglycemic rat can be seen on Figure 2 and Table 3. The percentage of wound closure of group P1 and P2 on the day 5 were lower than that of group K2. However, wound closure of P2 was higher than K2 on the day 10. The wound closure on

Table 2: Grouping and Treatment of Animals.

Group	Remarks
P1	Diabetic experimental rats treated with 20 µg aucubin
P2	Diabetic experimental rats treated with 40 µg aucubin
P3	Normal control rats treated with 40 µg aucubin
K1	Positive control treated with bioplacenton [®] gel
K2	Negative control treated with gel base

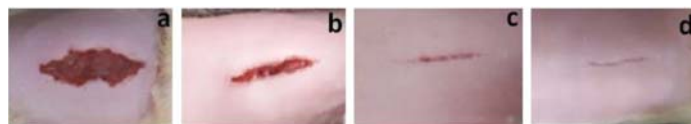


Figure 2: Development of wound on hyperglycemic rats treated with aucubin gel from day of 0 (a), 5 (b), 10 (c), and 21 (d).

Table 3: Effect of Aucubin on Wound Closure of Hyperglycemic Rats.

Group	Wound closure (%) [*]		
	Day 5	Day 10	Day 21
P1	18.45 \pm 2.19 ^c	58.08 \pm 11.59	100.00 \pm 0.00
P2	17.86 \pm 3.02 ^c	91.86 \pm 7.88 ^a	100.00 \pm 0.00
P3	31.71 \pm 3.62 ^b	94.86 \pm 11.49	100.00 \pm 0.00
K1	27.28 \pm 2.81	44.12 \pm 8.78 ^b	100.00 \pm 0.00
K2	22.56 \pm 4.04 ^{a,b,c}	44.72 \pm 8.79 ^{b,c}	83.47 \pm 7.80 ^{a,b,c}

^{*}data represent the means \pm SD of 5 replications; ^a = $p < 0.05$ compared to P1; ^b = $p < 0.05$ compared to P2; ^c = $p < 0.05$ compared to K1.

Table 4: Effect of Aucubin on Wound Healing Time of Hyperglycemic Rats.

Group	Wound healing time (day) [*]
P1	12.80 \pm 1.30 ^{b,c}
P2	10.60 \pm 0.55 ^c
P3	10.00 \pm 0.70
K1	19.00 \pm 1.58
K2	24.40 \pm 1.14 ^{a,b,c}

^{*}data represent the means \pm SD of 5 replications; ^a = $p < 0.05$ compared to P1; ^b = $p < 0.05$ compared to P2; ^c = $p < 0.05$ compared to K1.

the day 21 exhibit a significant difference between group K2 with P1, P2, P3, and K1.

Effect of aucubin on the wound healing time

Effect of aucubin on the wound healing time of hyperglycemic rat can be seen on Table 4. The wound healing time of P2, group treated with 40 µg aucubin, was significantly shorter than P1 and K1.

DISCUSSION

Various herbs have been studied for their wound healing activity, such as: *Plantago major*, *Carica papaya*, *Malva sylvestris*, *Terminalia chebula*, *Chrysophyllum cainito*, *Achyranthes aspera*, *Azadirachta indica*.¹⁰⁻¹⁷ Wound healing is a complex process involving a variety of cellular and matrix components acting in concert to reestablish the integrity of the injured tissue. The complexity of the healing response can be simplified into four broad categories that coincide with the temporal sequence of normal healing: hemostasis, inflammation, cell proliferation and tissue

remodeling. After tissue injury, red blood cells and platelets aggregate and form an initial hemostatic plug to protect the wound. Within 24 h, neutrophils enter the wound site and scavenge cellular debris, foreign bodies and bacteria. After 2-3 days, the inflammatory cell population begins to shift to macrophages and fibroblasts appear in the wound site. After 3-5 days, the fibroblasts become activated and begin synthesizing collagen. As the collagenous matrix forms, densely packed fibers fill the wound site and during remodeling, the wound gradually becomes stronger with time.⁹ However, hyperglycemic conditions in diabetic foot ulcer slow down this healing process.

Previous studies have reported that aucubin may exert its anti-inflammatory effects by inhibiting Ag-induced TNF- α and IL-6 production and expression by blocking NF- κ B activation in RBL-2H3 mast cells.¹⁸ In this study aucubin was prepared into gel dosage form. This dosage form has several advantages such as: clear appearance, high adhesion, spread easily, easy to clean, making skin cool and comfort.¹⁹

The wound closure of hyperglycemic rats treated with aucubin (20 and 40 μ g) were lower than that of negative control on day 5. It means that within five days both 20 and 40 μ g of aucubin could not improve the wound healing process. However, wound closure of animal group treated with aucubin (40 μ g) was higher than negative control on the day 10. This indicated that within ten days aucubin in the dose of 40 μ g could facilitate the wound healing process. Normal rats (group P3) were used to ensure that aucubin was able to treat incision wounds under non-hyperglycemic conditions as well. However, statistical analysis showed that the wound closure of hyperglycemic rats (group P2) and normal rats (group P3) were not significantly different on the day 10. This may occur because the wound healing activity of aucubin in hyperglycemic conditions is as strong as in non-hyperglycemic conditions. Moreover, the wound closure of hyperglycemic rats treated with 40 μ g of aucubin (group P2) was significantly higher than that of group received 20 μ g of aucubin (group P1) on the day 10. This indicated that the higher dose, the higher activity. However, a dermal toxicity test of aucubin is further required to ensure its safety.

Both aucubin and positive control exhibited complete wound closure on the day 21. The proliferative phase is the phase in which fibroblasts undergo proliferation and synthesize collagen, thus forming collagen fibers which will close the wound.²⁰ This phase usually occurs up to 21 days, therefore the wound closure was analyzed during 21 days.

The time taken by the wound to close perfectly was evaluated as well. Wound healing time of group treated with 40 μ g aucubin was significantly shorter than those of 20 μ g of aucubin and positive control. This is consistent with the result of wound closure. At the higher dose, aucubin has the higher percentage of wound closure and shorter healing time. Wound healing time of normal rats treated with aucubin were not different from the hyperglycemic rats. This also indicated that wound healing activity of aucubin in hyperglycemic conditions is as strong as non-hyperglycemic conditions. Zheng *et al.* showed that aucubin may inhibit the growth of *Escherichia coli* and *Staphylococcus aureus*, while wounds in hyperglycemic conditions are particularly susceptible to gram-positive bacteria such as *Staphylococcus*, *Streptococcus*, and *Clostridium*.⁶ The wound healing time of vehicle group (group K2) was the longest amongst the other groups. This is also consistent with the result of wound closure on day 21, which is base gel as a negative control could not accelerate wound healing in hyperglycemic conditions.

CONCLUSION

Aucubin gel at doses of 20 μ g and 40 μ g were able to increase the wound closure and shorten the wound healing time in hyperglycemic rats. Therefore, aucubin can be used as a drug candidate for diabetic wound healing.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

There are no conflicts of interest.

ABBREVIATIONS

DFU: Diabetic Foot Ulcer; **CMC-Na:** Carboxymethyl cellulose; **Ag:** Antigen; **TNF- α :** Tumor Necrosis Factor Alpha; **IL-6:** Interleukin 6; **NF- κ B:** Nuclear Factor kappa-light-chain-enhancer of activated B cells; **RBL-2H3:** Rat Basophilic Leukemia.

SUMMARY

- *Plantago major* has been used empirically for wound healing.
- Aucubin, an iridoid glycosides, is widely distributed in plants including *Plantago major*.
- Topical gel containing aucubin at the dose of 20 μ g and 40 μ g were able to increase the percentage of wound closure and accelerate wound healing time on hyperglycemic rat.
- Aucubin can be used as a drug candidate for diabetic wound healing.

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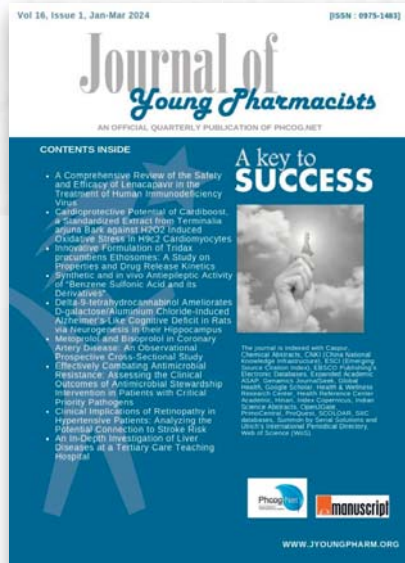
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Email: editor@youngpharm.org
Support: journals@phcoo.net



Journal of Young Pharmacists

Discontinued in Scopus as of 2018

<p>COUNTRY</p> <p>India</p> <div style="background-color: #333; color: white; padding: 5px; margin-bottom: 5px;">  Universities and research institutions in India </div> <div style="background-color: #333; color: white; padding: 5px;">  Media Ranking in India </div>	<p>SUBJECT AREA AND CATEGORY</p> <p>Pharmacology, Toxicology and Pharmaceutics</p> <ul style="list-style-type: none"> └ Pharmacology, Toxicology and Pharmaceutics (miscellaneous) 	<p>PUBLISHER</p> <p>EManuscript Technologies</p>	<p>H-INDEX</p> <p style="font-size: 2em; font-weight: bold;">36</p>
<p>PUBLICATION TYPE</p> <p>Journals</p>	<p>ISSN</p> <p>09751483, 09751505</p>	<p>COVERAGE</p> <p>2010-2018, 2021</p>	<p>INFORMATION</p> <p>Homepage</p> <p>How to publish in this journal</p> <p>editor@jyoungpharm.org</p>

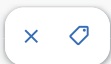
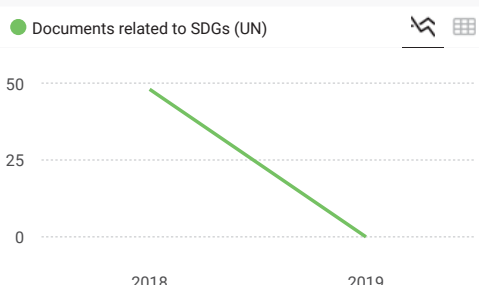
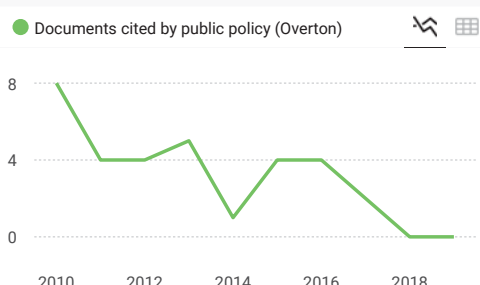
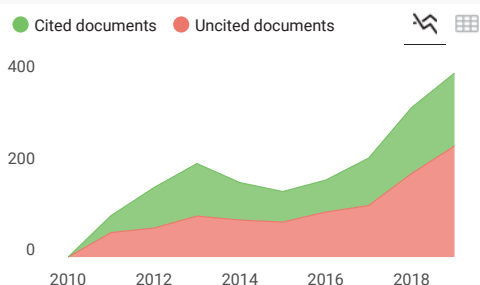
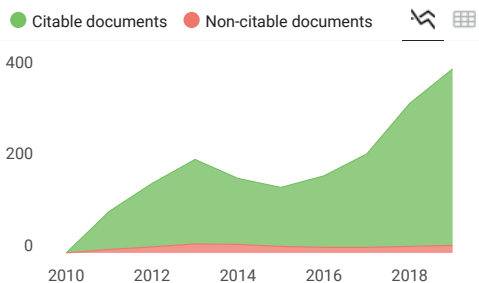
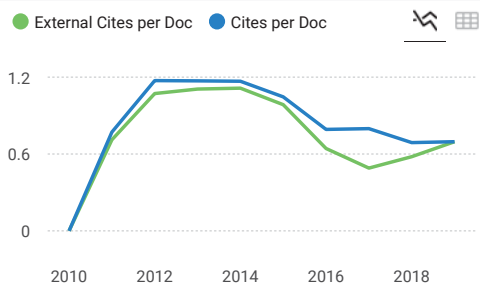
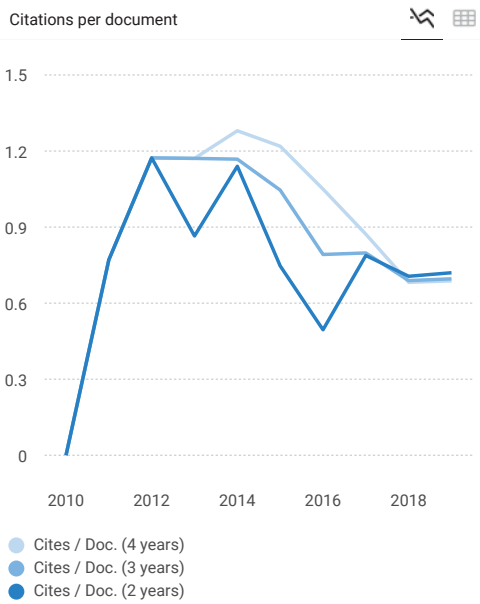
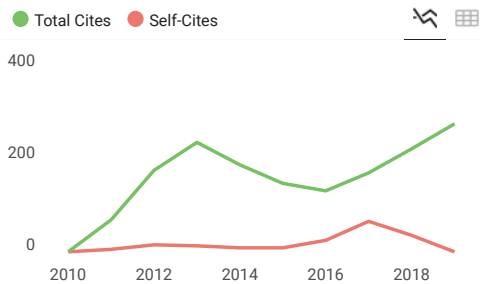
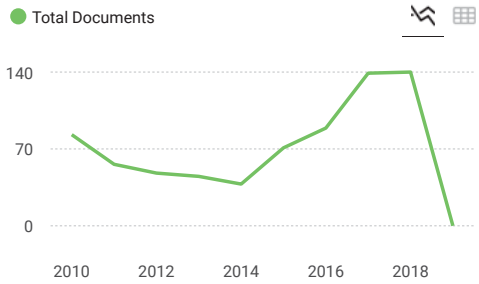
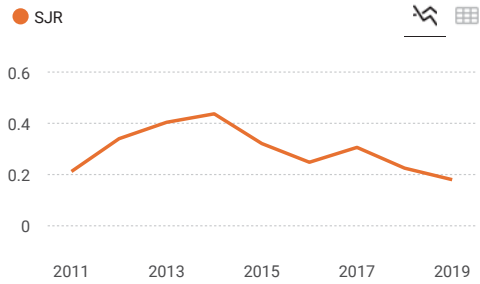
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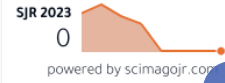






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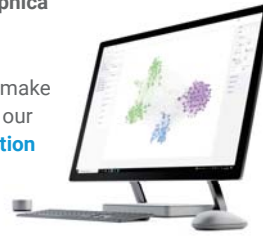
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Ahmad Najib 4 years ago

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Najib Ahmad 4 years ago

Thank you very much for your information



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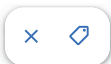
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Ainur Rohmah 5 years ago

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