



Yunita Sari <sasa.yunita@gmail.com>

Sending the submission of full-text for publication in IOP

Yunita Sari <sasa.yunita@gmail.com>

Sat, Nov 7, 2020 at 4:28 PM

To: ICMA SURE 2020 Committee <icmasure.lppm@unsoed.ac.id>

Dear ICMA-SURE Committee,

I would like to submit the full text of my manuscript which I already presente in ICMA-SURE 2020 for publication in IOP. Please find it in the attachment.

Sincerely,
Yunita



Submission A Comparison of The Effectiveness Banana Stem Sap and Virgin Coconut Oil on Diabetic Wound Healing.docx

1861K



Yunita Sari <sasa.yunita@gmail.com>

Submission of article 1456

ICMA SURE 2020 Committee <icmasure.lppm@unsoed.ac.id>

Mon, Nov 9, 2020 at 8:02 AM

To: Yunita Sari <sasa.yunita@gmail.com>

Dear Author,

We have received your submission. We will send it to the reviewers and send the reviewer comments to you.

Thank you.

Best regards,
ICMA-ICLASSURE 2020
Institute of Research and Community
Service Jenderal Soedirman University



Yunita Sari <sasa.yunita@gmail.com>

Review of article 1456

ICMA SURE 2020 Committee <icmasure.lppm@unsoed.ac.id>

Thu, Nov 19, 2020 at 10:46 PM

To: Yunita Sari <sasa.yunita@gmail.com>

Dear Author,

We have received feedback from the reviewers. Here I summary the reviewers comments. Please make the revision as soon as possible before November 26, 2020.

Thank you,

ICMA-ICLASSURE 2020
Institute of Research and Community Service
Jenderal Soedirman University

First reviewer :

1. The study has original point
2. Introduction: the reason to study banana stemsap dan vco need to be explained
3. Where the VCO and BSS are obtained? it should be described in the method
4. How much of the extract was applied to thewound? It should be described in the method
- 5.Method: how to measrure wound size. It should be described in the method

Second reviewer :

1. Add the p-value in the differences of the intensity of PMN staining and wound size
2. Add limitation of the study
3. Add the implication in clinical practice



Yunita Sari <sasa.yunita@gmail.com>

Sending a revised file and Certificate of English Edit

Yunita Sari <sasa.yunita@gmail.com>

Wed, Nov 25, 2020 at 5:04 PM

To: ICMA SURE 2020 Committee <icasure.lppm@unsoed.ac.id>

Dear ICMA-SURE Committe,

Thank you very much for the review of article. We have revised all the comments of reviewers. We hope our article is suitable for publication in IOP. Here I send the revised version, author comments, and certificate of English Editing. please let me know if there are other comments from reviewers.

Thank you,
Yunita

3 attachments



Author Responses of Review.docx

18K



Certification.docx

1022K



revised version A Comparison of The Effectiveness Banana Stem Sap and Virgin Coconut Oil on Diabetic Wound Healing.docx

1863K

Author Responses of Review

First reviewer :

1. The study has original point
2. Introduction: the reason to study banana stemsap dan vco need to be explained
3. Where the VCO and BSS are obtained? it should be described in the method
4. How much of the extract was applied to the wound? It should be described in the method
5. Method: how to measure wound size. It should be described in the method

Second reviewer :

1. Add the p-value in the differences of the intensity of PMN staining and wound size
2. Add limitation of the study
3. Add the implication in clinical practice

Answers :

Questions from 1st reviewer

1. Thank you for your comment
2. Thank you, we have added as suggested. We added the below sentences. The revision is in red.
**The herbals and natural products exert a promising effects in the wound management since many of them can facilitate disinfection, debridement, and provide a moist environment. Natural products, which are widely used in Indonesia rural areas to treat wounds, are banana stem sap (BSS) and virgin coconut oil (VCO)[3].
The wound size, the presence of necrotic tissue, level of inflammation, level of fibroblast, and re-epithelialization were assessed**
3. Thank you. We added the sentences in the method about where we obtained BSS and VCO. The revision is in red
The VCO used in this study was commercially available and purchased from the small industry CV Al Afiat, and was approved by the local authority of BPOM, the Indonesia FDA-like agency. This product was 100% VCO and was ready to use. The BSS used in this study was purchased from a local shop. The processing was performed at the Department of Pharmacy, Universitas Jenderal Soedirman, Purwokerto, Indonesia.
4. Thank you. We added the sentences in the method section as suggested. The sentences added are as follow:
In the BSS and VCO groups, 50 µL corresponding extract was applied.
5. Thank you. We added the sentences in the method section as suggested, and add the reference. the sentences added are as follow:
Relative wound area was calculated as follows: $\text{size at day } n - \text{size at day } 0 / (\text{size at day } 0)$. [14]

Answers of Questions from 2nd Reviewer :

1. Thank you, we have added the p-value in the result section. the sentences added are below

However, from day 9 onwards, wounds in the BSS group were significantly smaller compared with the ones in the VCO group (day 9, $p=0.034$; day 10, $p=0.034$, day 11, $p=0.34$, day 12, $p=0.034$, day 13, $p=0.034$, and day 14, $p=0.034$) and control group (day 9, $p=0.043$; day 10, $p=0.043$, day 11, $p=0.042$, day 12, $p=0.042$, day 13, $p=0.021$, day 14, $p=0.021$).

However, from day 10 onwards, wounds in the control group were significantly smaller compared with the ones in the VCO group (day 10, $p=0.034$; day 11, $p=0.032$, day 12, $p=0.032$, day 13, $p=0.034$, day 14, $p=0.034$).

The result of HE staining on day 14 showed that the intensity of PMNs in BSS group was not only significantly lower compared with control group ($p = 0.008$), but also compared with VCO group ($p = 0.008$).

2. Thank you, we have added the limitation of the study as suggested. the revision are as follow

This study has one limitation. We investigated the wound healing effect only in term of inflammation, and reepithelialization. However, our study is the first comparing BSS and VCO effect in the healing of diabetic ulcers consecutively and therefore the progression of wound healing was thoroughly observed.

3. Thank you, we have added the implication in the clinical setting. the added sentences are below. The revision is in red

Taken together, our study indicated that BSS was more effective in promoting the healing of diabetic ulcers, and therefore it might be a good candidate in the clinical usage. Nurse and clinician should use BSS over VCO to treat diabetic ulcers. A further study on human subject with diabetic ulcers is needed to confirm these findings.



CERTIFICATE OF ENGLISH EDITING

This is to certify that the manuscript entitled
**A Comparison of The Effectiveness Banana Stem Sap and Virgin Coconut Oil
on Diabetic Wound Healing**

commissioned to us has been carefully edited by a native English-speaking editor of MogoEdit, and the grammar, spelling, and punctuation have been verified and corrected where needed. Based on this review, we believe that the language in this paper meets academic journal requirements. Please contact us with any questions.



Gang Zhang

Dr. Gang Zhang
Founder & CEO of MogoEdit

Date of Issue
November 24, 2020

Disclaimer: The changes in the document may be accepted or rejected by the authors in their sole discretion after our editing. However, MogoEdit is not responsible for revisions made to the document after our edit on November 24, 2020.

MogoEdit is a professional English editing company who provides English language editing, translation, and publication support services to individuals and corporate customers worldwide. As a company invested by the affiliate fund of Chinese Academy of Science, MogoEdit is one of the leading language editing service providers in China, whose clients come from more than 1000 universities and research institutes.

MogoEdit Website: <http://en.mogoedit.com/>

500+ native English editors: <http://en.mogoedit.com/editors>



Mogo Internet Technology Co., LTD.

No. 25, 1st Gaoxin Road, Xi'an 710075, PR China +86 02988317483 support@mogoedit.com

Revised Manuscript

A Comparison of The Effectiveness Banana Stem Sap and Virgin Coconut Oil on Diabetic Wound Healing

Y. Sari¹, A. Isworo¹, A. S. Upoyo¹, A. Sumeru¹, D. W. Kurniawan², E. Sutrisna³

¹ Department of Nursing, Faculty of health sciences, Universitas Jenderal Soedirman, Purwokerto, 53123 Indonesia

² Department of Pharmaceutics, Universitas Jenderal Soedirman, Purwokerto, 53125 Indonesia

³ Department of Pharmacology, Faculty of medicine, Universitas Jenderal Soedirman, Purwokerto, 53123 Indonesia

E-mail address: sasa.yunita@gmail.com/ yunita.sari@unsoed.ac.id

Abstract. Banana stem sap (BSS) and virgin coconut oil (VCO) are usually used for wound care treatment. However, to date, no study has been conducted to compare the effectiveness of these two therapies. Therefore, the aim of this study was to compare the effectiveness of BSS and VCO on diabetic wound healing in rat. Male Wistar rats were randomly divided into 3 groups depending on the formulation used on the wound: BSS, VCO, and control treated with film dressing. The tissue samples were obtained on day 4, and 14. **The wound size, the presence of necrotic tissue, level of inflammation, level of fibroblast, and re-epithelialization were assessed.** On day 10, wounds in the BSS group were the smallest compared with other groups. The necrotic tissue and level of inflammation in the BSS group were also less as compared with other groups. The reepithelialization in BSS group was also the most advance compared with other groups. Our study showed that the ability of BSS in accelerating wound healing is better than VCO and film dressing. We recommend nurses and clinician in Indonesia to use BSS to treat diabetic ulcer.

1. Introduction

The number of patients with diabetes mellitus (DM) in the world, including Indonesia, has become alarming because of its dramatic increase. It has been predicted that Indonesia will reach the sixth place with regard to the number of people with DM in the world in 2030. The increase of people with DM will also cause an increase of DM complications, especially diabetic ulcers. A previous study showed that approximately 26,1 million people with DM suffer from diabetic ulcers. Diabetic ulcers can become infected, leading to invasive systemic infection and mortality. Indeed, the 5 year mortality rate from diabetic ulcer is 74%. The highest mortality rate due to diabetic ulcer occurs in low and middle income countries [1].

In order to improve the wound healing, advanced wound care technology is usually used for diabetic ulcer [2]. However, the advanced technology is usually rare in Indonesia. Therefore, many herbals and natural products are often used as a wound healer. **The herbals and natural products exert a promising effects in the wound management since many of them**

can facilitate disinfection, debridement, and provide a moist environment. Natural products, which are widely used in Indonesia rural areas to treat wounds, are banana stem sap (BSS) and virgin coconut oil (VCO)[3].

Banana plants usually grow in south east Asia, including Indonesia.[4] They contain many beneficial factors such as catecholamine, iron, vitamin B and C, albuminoids, fats, and mineral salts [4–6]. Banana stem sap (BSS) is the part of banana plant that shows most of the beneficial effects. Indeed, BSS contains the mentioned factors plus also flavonoids, saponins, and tannins that work as antioxidants, and they exert antibacterial, antifungal, and pain relief effect [4]. In addition, BSS contains lectin that stimulates skin cell growth (angiogenesis) [7]. As regard the wound healing process, a study of Priosoeryanto et al (2007) in an acute wound model showed that BSS can reduce inflammation in an incision wound and improve the number of fibroblasts and the amount of granulation tissue [8].

VCO has been widely used to treat microbial infections and skin problems and in eastern Indonesia to treat wounds [9]. VCO contains many antioxidants such as polyphenols, tocopherol, ferulic acid, phenolic acid and flavonoids, and also vitamin E [10]. VCO also has antimicrobial properties [9]. As regard wound healing, VCO can reduce inflammation in palatoplasty surgical wound and VCO flavonoids can improve wound reepithelialization, increase anti-oxidant level, and increase the amount of granulation tissue[11]. Moreover, VCO can reduce infection [9] .

According to the aforementioned properties of BSS and VCO, both these topical therapies could be candidate for wound care treatment. However, it is also important to evaluate which of these two therapies is more effective in promoting wound healing, since no study is available that directly compared these two plant extracts. Therefore, the purpose of this study was to compare the effectiveness of BSS and VCO in the promotion of diabetic ulcer healing.

2. Materials and methods

2.1. Animals

Healthy Male Wistar Rats (180-200 grams) were obtained from the Laboratory of animal, Department of Pharmaceutical, Universitas Muhammadiyah Purwokerto, Indonesia. Rats were housed in one cage per rat and they have free access to food and drink ad libitum. Our study protocol was approved by the ethical committee of the Faculty of Medicine, Universitas Jenderal Soedirman, Indonesia.

2.2. Herbal extracts

The VCO used in this study was commercially available and purchased from the small industry CV Al Afiat, and was approved by the local authority of BPOM, the Indonesia FDA-like agency. This product was 100% VCO and was ready to use. The BSS used in this study was purchased from a local shop. The processing was performed at the Department of Pharmacy, Universitas Jenderal Soedirman, Purwokerto, Indonesia.

The preparation of the BSS was performed according to previous study with some modifications.[12,13] In brief, the outer green part of the banana stem was peeled off and the inner portion was cut into small pieces. The pieces were then crushed and approximately 100 ml juice was extracted. The obtained liquid was applied to the wound using a swab. Every day of wound treatment, the remaining of liquid was freshly made, the remaining of liquid was thrown away.

2.3. Induction of diabetic rats and experimental procedure

Rats were injected with alloxan monohydrate 90 mg/Kg of body weight to induce diabetes mellitus based on previous study [14]. The glucose level higher than 275 mg/dl was considered diabetes mellitus. The rat was anesthetised with ketamine hydrochloride (25-30 mg/kg body weight). The wounding procedure was conducted based on the previous study. [15] In brief, one wound of 1 cm in size was created on the rat back dorsal region. Diabetic rats were divided into 3 groups depending on the formulation used on the wound: (1) BSS, (2) VCO (3) and control treated with film dressing. **In the BSS and VCO groups, 50 µL corresponding extract was applied.** The wounds were cleansed every day. During wound cleaning, the wound condition were recorded.

2.4. Wound areas

ImageJ software was used to measure wound areas. [16] **Relative wound area was calculated as follows: size at day n – size at day 0)/(size at day 0).**[14] Rats were sacrificed by overdose of anesthesia. The wound tissue along with surrounding normal skin was harvested on day 4, and 14 and fixed in 10% formalin for histological analysis.

2.5. Hematoxylin and eosin (HE) staining

The tissue immersed in formalin 10% underwent a series of dehydrations in alcohol and xylene and embedded in paraffin. Next, the tissue was cut into 4 µm thick slices using a microtome. Slices were deparaffinized, rehydrated in xylene and alcohol, and stained with HE. After mounting, sections were observed and analyzed under a microscope. The intensity of polymorphonuclear neutrophils (PMNs) and fibroblast was assessed based on previous study [51].

2.6. Statistical analysis

Statistical analysis was performed using SPSS 21 software package. Wound size was expressed as mean \pm standard deviation (SD). Differences in wound size, fibroblast intensity and inflammation intensity among groups were analyzed by ANOVA or Kruskal Wallis test. If a significant difference was found, post-hoc Tukey's HSD test or Mann-Whitney U-test was used. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Wound bed appearance

The wound bed appearance is shown in Figure 1. On day 1, the wound appearance was red in all groups, and no slough appeared. On day 3, the wound bed in both BSS and VCO group did not have slough, while a thin slough appear in the wounds of the control group, but the wound bed in the BSS group was redder compared with the one of the VCO group. On day 5, the wound bed in all groups was covered by a thin slough. On day 11, the slough was disappeared in the BSS group, while the wound bed in VCO and control group was still covered by slough. On day 14, the wounds in BSS group were almost healed and were covered with new epithelium, while in the VCO group, wounds were still covered with slough. The wound in the control group on day 14 showed no necrotic tissue and the wound bed was filled with granulation tissue.

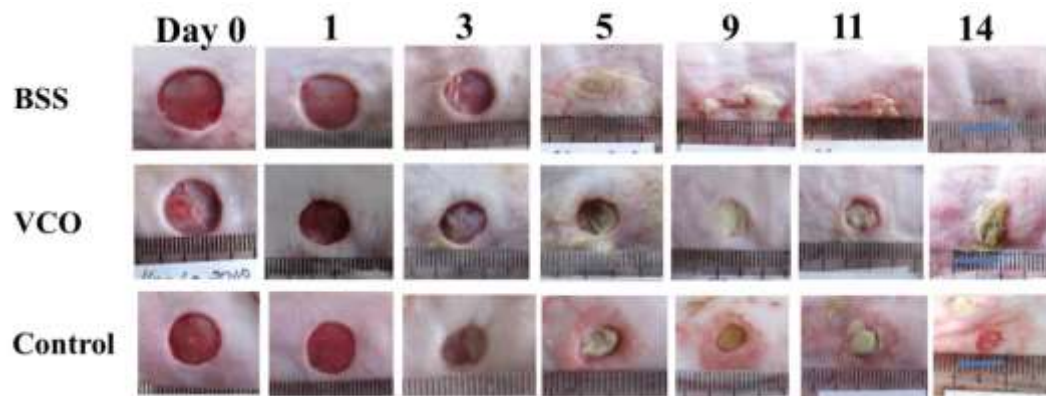


Figure 1. Wound bed appearance

There is no difference in the wound size among the three groups on inflammation phase and early proliferative phase. However, from day 9 onwards, wounds in the BSS group were significantly smaller compared with the ones in the VCO group (day 9, $p=0.034$; day 10, $p=0.034$, day 11, $p=0.34$, day 12, $p=0.034$, day 13, $p=0.034$, and day 14, $p=0.034$) and control group (day 9, $p=0.043$; day 10, $p=0.043$, day 11, $p=0.042$, day 12, $p=0.042$, day 13, $p=0.021$, day 14, $p=0.021$). On day 9, no significant difference was observed between control and VCO group regarding wound size. However, from day 10 onwards, wounds in the control group were significantly smaller compared with the ones in the VCO group (day 10, $p=0.034$; day 11, $p=0.032$, day 12, $p=0.032$, day 13, $p=0.034$, day 14, $p=0.034$).

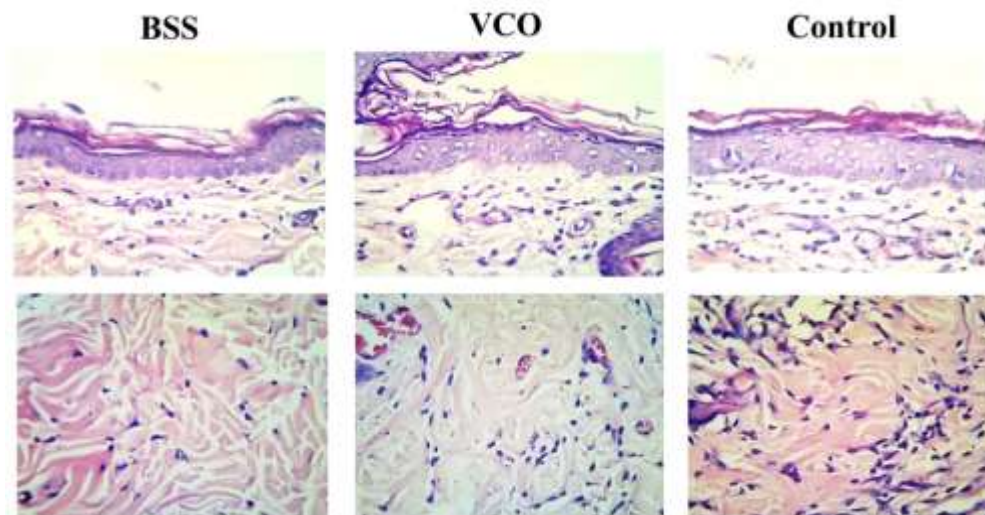


Figure 2. HE staining of epidermis (upper picture) and dermis (lower picture) in the BSS, VCO, and control group on day 4.

3.2. He staining

The result of HE staining in epidermis and dermis layer on day 4, and 14 are shown in figure 2, and 3, respectively. Figure 2 shows that the intensity of polymorphonuclear (PMNs) in BSS group was slightly lower than that in control group and VCO groups. The inflammation

in the VCO group was also slightly lower than that in control group. Figure 2 also shows that no significant difference was found in the intensity of fibroblast among the three groups on day 4. The result of HE staining on day 14 showed that the intensity of PMNs in BSS group was not only significantly lower compared with control group ($p = 0.008$), but also compared with VCO group ($p = 0.008$). In addition, the level of PMNs in the VCO group was significantly higher than that in the control group ($p = 0.008$). On day 14, the intensity of fibroblast in BSS group was significantly higher compared with control ($p = 0.008$) and VCO group ($p = 0.04$) (Table 1). Figure 3 also shows that the reepithelialization was most advanced in the BSS groups compared with the other groups.

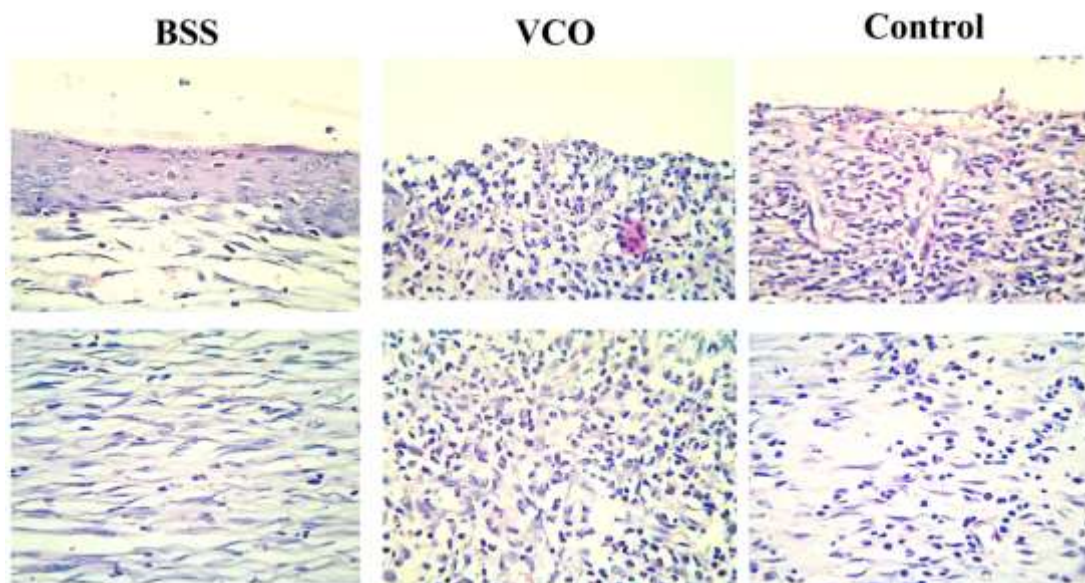


Figure 3. HE staining of epidermis (upper picture) and dermis (lower picture) in the BSS, VCO and control group on day 14.

Table 1. The difference in the intensity of PMNs and Fibroblast in each group on day 14

Groups	PMNs	Fibroblast
BSS	1*†	3*†
VCO	3*	2
Control	2	2

Values represent the median score. Scores can range from 0 to 4 (0 = absent; 1 = occasional; 2 = moderate; 3 = abundant; 4 = very abundant). * $p < 0.05$ (BSS/VCO vs control), † $p < 0.05$ (BSS vs VCO)

4. Discussion

This study is the first comparing BSS and VCO effect in the healing of diabetic ulcers. Our study indicated that wounds treated with BSS healed faster compared with the ones treated with VCO, even compared with the ones treated with use of modern wound-dressing film dressing.

One characteristic of diabetic wounds is a prolonged inflammation due to local hypoxia and bacterial burden. [17] The reduction of inflammation in BSS might be due to the fact that BSS contain anti-inflammatory, antioxidant and antibacterial agents.[4] A recent study

investigated ABSS composition, and found that BSS contains antioxidant such as polyphenols, tannin, saponins, and flavonoids. [12] In addition flavonoids have antibacterial activity. Indeed, the extract of banana stem has an antibacterial effect against staphylococcus aureus and pseudomonas aeruginosa.[18]

In this study, the slough in VCO group was thicker than in the other groups. Slough reduction in the BSS and control group might be due to the fact that BSS and film dressing exerted an autolytic debridement, resulting in thinner slough compared with VCO group. In the VCO group, the slough was thicker from day 5 to day 14. The presence of slough causes more extensive inflammation into the surrounding skin, resulting in more tissue damage and accumulation of dead tissue in the wound bed. [17] This might be the reason why the inflammation in the VCO group was higher and more extensive than the other groups on day 14. Our study is not in agreement with a previous study revealing that wounds treated with VCO healed better compared with the ones in the control group. [19] This discrepancy might be due to the fact that in the previous study, the healing ability of a wound treated with VCO was compared with healing ability of an untreated wound, while in this study VCO healing effect was compared with the effect exerted by the modern wound-dressing used in the control group. Film dressing provides a moist environment in the wound, therefore promoting its reepithelialization.[20]

In this study, BSS also improved the granulation tissue, in agreement with previous studies showing that BSS can improve granulation tissue formation.[21] The formation of granulation tissue is better in BSS than in VCO group and control group. In our study, the level of fibroblast was higher than that in the control and VCO group. Fibroblasts represent the basic materials of collagen (mucopolysaccharides, aminoglysin acid and proline), which is important for granulation tissue formation.[22] A recent study suggested that the mechanism involved in the improvement of the granulation tissue formation may be due to the ability of BSS to increase PDGF-BB.[21] PDGF stimulates fibroblast chemotaxis activity and proliferation, and the production of glycosaminoglycans. Glycosaminoglycans form a gel-like ground substance, in which the collagen fibers are embedded.

This study has one limitation. We investigated the wound healing effect only in term of inflammation, and reepithelialization. However, our study is the first comparing BSS and VCO effect in the healing of diabetic ulcers consecutively and therefore the progression of wound healing was thoroughly observed.

Taken together, our study indicated that BSS was more effective in promoting the healing of diabetic ulcers, and therefore it might be a good candidate in the clinical usage. Nurse and clinician should use BSS over VCO to treat diabetic ulcers. A further study on human subject with diabetic ulcers is needed to confirm these findings.

5. Conclusions

This study compared the effect of BSS, VCO and modern dressing on wound healing of diabetic rats. Our results showed that the inflammation and the presence of slough were the least in BSS group compared with VCO and control group. The reepithelialization was also most advanced in the BSS group. According to our evidences, BSS might be a good candidate for topical treatments of diabetic ulcer.

References

- [1] Shaw J E, Sicree R A and Zimmet P Z 2010 Global estimates of the prevalence of diabetes for 2010 and 2030 *Diabetes Res. Clin. Pract.* **87** 4–14

- [2] Khamaisi M and Balanson S 2017 Dysregulation of wound healing mechanisms in diabetes and the importance of negative pressure wound therapy (NPWT). *Diabetes. Metab. Res. Rev.* **33**
- [3] Haesler E, Watts R, Rice J C K 2016 Local resource botanicals used in wound care *Wound Pr. Res* **24** 84-90.
- [4] Imam M Z and Akter S 2011 *Musa paradisiaca* L . and *Musa sapientum* L . : A Phytochemical and Pharmacological Review Mohammad Zafar Imam and Saleha Akter *J. Appl. Pharm. Sci.* **01** 14–20
- [5] SHANMUGAVELU K G and RANGASWAMI G 1962 Tryptophan and Indole Compounds in Banana Ovaries *Nature* **194** 775
- [6] Lewis D A and Shaw G P 2001 A natural flavonoid and synthetic analogues protect the gastric mucosa from aspirin-induced erosions. *J. Nutr. Biochem.* **12** 95–100
- [7] Peumans W J, Zhang W, Barre A, Houles Astoul C, Balint-Kurti P J, Rovira P, Rouge P, May G D, Van Leuven F, Truffa-Bachi P and Van Damme E J 2000 Fruit-specific lectins from banana and plantain. *Planta* **211** 546–54
- [8] Priosoeryanto BP, Putriyanda N, AR L, V J, I W, BF P and R T 2007 The effect of ambon banana stem sap on the acceleration of wound healing process in mice (*Mus musculus albinus*) *J. Agric Rural Devp* 35–59
- [9] Sachs M, von Eichel J and Asskali F 2002 [Wound management with coconut oil in Indonesian folk medicine]. *Chirurg.* **73** 387–92
- [10] Marina A M, Man Y B C, Nazimah S A H and Amin I 2009 Antioxidant capacity and phenolic acids of virgin coconut oil. *Int. J. Food Sci. Nutr.* **60 Suppl 2** 114–23
- [11] Nevin K G and Rajamohan T 2010 Effect of topical application of virgin coconut oil on skin components and antioxidant status during dermal wound healing in young rats. *Skin Pharmacol. Physiol.* **23** 290–7
- [12] Hendrik Setia Budi, Indah Listiana Kriswandini and A D I 2015 Antioxidant activity test on ambonese banana stem sap (*Musa Dent. J.* **188** 188–92
- [13] Weremfo A and Adinortey M B 2011 Haemostatic Effect of the Stem Juice of *Musa paradisiaca* L . (*Musaceae*) in Guinea Pigs *Adv. Biol. Res. (Rennes)*. **5** 190–2
- [14] Sari Y, Hartono, Sutrisna E and Saryono 2019 The effect of short duration of electrical stimulation on wound healing in acute wound in a rat model *Wound Med.* **24** 36–44
- [15] Sari Y, Purnawan I, Kurniawan D W and Sutrisna E 2018 A Comparative Study of the Effects of *Nigella sativa* Oil Gel and Aloe Vera Gel on Wound Healing in Diabetic Rats. *J. evidence-based Integr. Med.* **23** 2515690X18772804
- [16] WS R 1997 ImageJ: image processing and analysis in Java
- [17] Sood A, Granick M S and Tomaselli N L 2014 Wound Dressings and Comparative Effectiveness Data *Adv. wound care* **3** 511–29
- [18] Amutha K and Selvakumari U 2016 Wound healing activity of methanolic stem extract of *Musa paradisiaca* Linn. (Banana) in Wistar albino rats. *Int. Wound J.* **13** 763–7
- [19] Soliman A M, Lin T S, Ghafar N A and Das S 2018 Virgin coconut oil and diabetic wound healing : histopathological and biochemical analysis *Eur. J. Anat* **22** 135–44
- [20] Diegelmann R F and Evans M C 2004 Wound healing: an overview of acute, fibrotic and delayed healing. *Front. Biosci.* **9** 283–9
- [21] Hs B, Il K and Sa S 2016 Ambonese banana stem sap (*Musa paradisiaca* var . *sapientum*) effect on PDGF-BB expressions and fibroblast proliferation in socket wound healing *Int. J. ChemTech Res.* **9** 558–64

- [22] Werner S and Grose R 2003 Regulation of wound healing by growth factors and cytokines. *Physiol. Rev.* **83** 835–70



Yunita Sari <sasa.yunita@gmail.com>

Article Revision Request 1456

ICLAS SURE LPPM <icmasure.lppm@unsoed.ac.id>
To: sasa.yunita@gmail.com

Thu, Feb 18, 2021 at 2:08 PM

Dear Yunita Sari, Atyanti Isworo, Arif Setyo Upoyo, Annas Sumeru, Dhadhang Wahyu Kurniawan, Eman Sutrisna

We are pleased to inform you that our conference has made agreement with IOP Conference Series: Earth and Environmental Science (EES). The selected paper which passing the review process will be published in EES IOP Conference Series. At this moment, your article entitled **A Comparison of The Effectiveness Banana Stem Sap, Virgin Coconut Oil on Diabetic Wound Healing** is in the 2nd round of review.

The review consisted of content review, layout review, and similarity check.

1. Please check your manuscript progress through Online Conference System (OCS) <https://conference.unsoed.ac.id/index.php/icma/ICLAS2020/login>
2. Please follow the reviewer's suggestion for the content which attached in OCS. In case you didn't find any attachment on your OCS, please contact us.
3. Please follow the EES IOP format based on this guideline <https://publishingsupport.iopscience.iop.org/author-guidelines-for-conference-proceedings/> or <https://drive.google.com/drive/folders/1ebshf4wl5LSn4vvmEiTtWAhYr3qcn0?usp=sharing>
4. Please use Mendeley and use IOP format. You may follow this video to add IOP Conference Series style on Mendeley <https://youtu.be/dtDoRmKb7VQ>
5. Please check the similarity using Turnitin and upload the results to the OCS along with the manuscript revision. The similarity must be less than 20%, otherwise your paper will be automatically rejected by EES IOP
6. Please return the revised article before 24 February 2021. We will not proceed the article which is not returned and revised. In case you don't want to continue this process, please inform us through this e-mail address.
7. The publication fee is IDR 1,500,000. The payment could be sent to BNI 40552249 (Christina Tri Setyorini). Please send a confirmation of your payment and send the transfer copy to this e-mail before 24 February 2021. We will not proceed the article if the author does not finish the payment. In case your paper does not pass the IOP standard reviewed by the IOP committee, we will return your payment.

Looking forward to hearing from you

ICMA-ICLASSURE 2020

Institute of Research and Community Service

Jenderal Soedirman University



Yunita Sari <sasa.yunita@gmail.com>

The content of 2nd review cannot be read

Yunita Sari <sasa.yunita@gmail.com>

Sat, Feb 20, 2021 at 6:14 PM

To: ICMA SURE 2020 Committee <icmasure.lppm@unsoed.ac.id>

Dear ICMA-SURE Committe,

Thank you for your email. However, I could not find the content of the 2nd review in the system. Could you please let me know the content of the 2nd review. Thank you

Sincerely,
Yunita Sari



Yunita Sari <sasa.yunita@gmail.com>

Article revision of 1456 (second review)

ICMA SURE 2020 Committee <icmeasure.lppm@unsoed.ac.id>

Sat, Feb 20, 2021 at 8:16 PM

To: Yunita Sari <sasa.yunita@gmail.com>

Dear Author,

I am sorry there is an error in the system. The content of 2nd review is that you need to adjust your manuscript according to the format guidelines. Please use font of 11 throughout the manuscript (except for abstract use font of 10). Please revise accordingly.

ICMA-ICLASSURE 2020
Institute of Research and Community ServiceJenderal
Soedirman University



Yunita Sari <sasa.yunita@gmail.com>

Article Revision Request 1456

Yunita Sari <sasa.yunita@gmail.com>

Tue, Feb 23, 2021 at 9:59 PM

To: ICLAS SURE LPPM <icmeasure.lppm@unsoed.ac.id>

Dear ICLAS SURE,

Here we attach the revised file, turnitin and also the receipt of payment

thank you
Yunita

On Thu, Feb 18, 2021 at 2:08 PM ICLAS SURE LPPM <icmeasure.lppm@unsoed.ac.id> wrote:

[Quoted text hidden]

3 attachments**receipt of payment.jpg**
44K**A Comparison of The Effectiveness Banana Stem Sap and Virgin Coconut Oil on Diabetic Wound Healing.docx**
1863K**turnitin A Comparison of The Effectiveness Banana Stem Sap and Virgin Coconut Oil on Diabetic Wound Healing (1).pdf**
2244K

A Comparison of The Effectiveness Banana Stem Sap and Virgin Coconut Oil on Diabetic Wound Healing

Y. Sari¹, A. Isworo¹, A. S. Upoyo¹, A. Sumeru¹, D. W. Kurniawan², E. Sutrisna³

¹Department of Nursing, Faculty of health sciences, Universitas Jenderal Soedirman, Purwokerto, 53123 Indonesia

²Department of Pharmaceutics, Universitas Jenderal Soedirman, Purwokerto, 53125 Indonesia

³Department of Pharmacology, Faculty of medicine, Universitas Jenderal Soedirman, Purwokerto, 53123 Indonesia

E-mail address: sasa.yunita@gmail.com/ yunita.sari@unsoed.ac.id

Abstract. Banana stem sap (BSS) and virgin coconut oil (VCO) are usually used for wound care treatment. However, to date, no study has been conducted to compare the effectiveness of these two therapies. Therefore, the aim of this study was to compare the effectiveness of BSS and VCO on diabetic wound healing in rat. Male Wistar rats were randomly divided into 3 groups depending on the formulation used on the wound: BSS, VCO, and control treated with film dressing. The tissue samples were obtained on day 4, and 14. **The wound size, the presence of necrotic tissue, level of inflammation, level of fibroblast, and re-epithelialization were assessed.** On day 10, wounds in the BSS group were the smallest compared with other groups. The necrotic tissue and level of inflammation in the BSS group were also less as compared with other groups. The reepithelialization in BSS group was also the most advance compared with other groups. Our study showed that the ability of BSS in accelerating wound healing is better than VCO and film dressing. We recommend nurses and clinician in Indonesia to use BSS to treat diabetic ulcer.

1. Introduction

The number of patients with diabetes mellitus (DM) in the world, including Indonesia, has become alarming because of its dramatic increase. It has been predicted that Indonesia will reach the sixth place with regard to the number of people with DM in the world in 2030. The increase of people with DM will also cause an increase of DM complications, especially diabetic ulcers. A previous study showed that approximately 26,1 million people with DM suffer from diabetic ulcers. Diabetic ulcers can become infected, leading to invasive systemic infection and mortality. Indeed, the 5 year mortality rate from diabetic ulcer is 74%. The highest mortality rate due to diabetic ulcer occurs in low and middle income countries [1].

In order to improve the wound healing, advanced wound care technology is usually used for diabetic ulcer [2]. However, the advanced technology is usually rare in Indonesia. Therefore, many herbals and natural products are often used as a wound healer. **The herbals and natural products exert a promising effects in the wound management since many of them can facilitate disinfection, debridement, and provide a moist environment. Natural products, which are widely used in Indonesia rural areas to treat wounds, are banana stem sap (BSS) and virgin coconut oil (VCO)[3].**

Banana plants usually grow in south east Asia, including Indonesia.[4] They contain many beneficial factors such as catecholamine, iron, vitamin B and C, albuminoids, fats, and mineral salts [4–6]. Banana stem sap (BSS) is the part of banana plant that shows most of the beneficial effects. Indeed, BSS contains the mentioned factors plus also flavonoids, saponins, and tannins that work as antioxidants, and they exert antibacterial, antifungal, and pain relief effect [4]. In addition, BSS contains lectin that stimulates skin cell growth (angiogenesis) [7]. As regard the wound healing

process, a study of Priosoeryanto et al (2007) in an acute wound model showed that BSS can reduce inflammation in an incision wound and improve the number of fibroblasts and the amount of granulation tissue [8].

VCO has been widely used to treat microbial infections and skin problems and in eastern Indonesia to treat wounds [9]. VCO contains many antioxidants such as polyphenols, tocopherol, ferulic acid, phenolic acid and flavonoids, and also vitamin E [10]. VCO also has antimicrobial properties [9]. As regard wound healing, VCO can reduce inflammation in palatoplasty surgical wound and VCO flavonoids can improve wound reepithelialization, increase anti-oxidant level, and increase the amount of granulation tissue [11]. Moreover, VCO can reduce infection [9].

According to the aforementioned properties of BSS and VCO, both these topical therapies could be candidate for wound care treatment. However, it is also important to evaluate which of these two therapies is more effective in promoting wound healing, since no study is available that directly compared these two plant extracts. Therefore, the purpose of this study was to compare the effectiveness of BSS and VCO in the promotion of diabetic ulcer healing.

2. Materials and methods

2.1. Animals

Healthy Male Wistar Rats (180-200 grams) were obtained from the Laboratory of animal, Department of Pharmaceutical, Universitas Muhammadiyah Purwokerto, Indonesia. Rats were housed in one cage per rat and they have free access to food and drink ad libitum. Our study protocol was approved by the ethical committee of the Faculty of Medicine, Universitas Jenderal Soedirman, Indonesia.

2.2. Herbal extracts

The VCO used in this study was commercially available and purchased from the small industry CV Al Afiat, and was approved by the local authority of BPOM, the Indonesia FDA-like agency. This product was 100% VCO and was ready to use. The BSS used in this study was purchased from a local shop. The processing was performed at the Department of Pharmacy, Universitas Jenderal Soedirman, Purwokerto, Indonesia.

The preparation of the BSS was performed according to previous study with some modifications.[12,13] In brief, the outer green part of the banana stem was peeled off and the inner portion was cut into small pieces. The pieces were then crushed and approximately 100 ml juice was extracted. The obtained liquid was applied to the wound using a swab. Every day of wound treatment, the remaining of liquid was freshly made, the remaining of liquid was thrown away.

2.3. Induction of diabetic rats and experimental procedure

Rats were injected with alloxan monohydrate 90 mg/Kg of body weight to induce diabetes mellitus based on previous study [14]. The glucose level higher than 275 mg/dl was considered diabetes mellitus. The rat was anesthetised with ketamine hydrochloride (25-30 mg/kg body weight). The wounding procedure was conducted based on the previous study. [15] In brief, one wound of 1 cm in size was created on the rat back dorsal region. Diabetic rats were divided into 3 groups depending on the formulation used on the wound: (1) BSS, (2) VCO (3) and control treated with film dressing. **In the BSS and VCO groups, 50 µL corresponding extract was applied.** The wounds were cleansed every day. During wound cleaning, the wound condition were recorded.

2.4. Wound areas

ImageJ software was used to measure wound areas. [16] **Relative wound area was calculated as follows: $\text{size at day } n - \text{size at day } 0 / (\text{size at day } 0)$.**[14] Rats were sacrificed by overdose of anesthesia. The wound tissue along with surrounding normal skin was harvested on day 4, and 14 and fixed in 10% formalin for histological analysis.

2.5. Hematoxylin and eosin (HE) staining

The tissue immersed in formalin 10% underwent a series of dehydrations in alcohol and xylene and embedded in paraffin. Next, the tissue was cut into 4 μm thick slices using a microtome. Slices were deparaffinized, rehydrated in xylene and alcohol, and stained with HE. After mounting, sections were observed and analyzed under a microscope. The intensity of polymorphonuclear neutrophils (PMNs) and fibroblast was assessed based on previous study [51].

2.6. Statistical analysis

Statistical analysis was performed using SPSS 21 software package. Wound size was expressed as mean \pm standard deviation (SD). Differences in wound size, fibroblast intensity and inflammation intensity among groups were analyzed by ANOVA or Kruskal Wallis test. If a significant difference was found, post-hoc Tukey's HSD test or Mann-Whitney U-test was used. A value of $p < 0,05$ was considered statistically significant.

3. Results

3.1. Wound bed appearance

The wound bed appearance is shown in Figure 1. On day 1, the wound appearance was red in all groups, and no slough appeared. On day 3, the wound bed in both BSS and VCO group did not have slough, while a thin slough appear in the wounds of the control group, but the wound bed in the BSS group was redder compared with the one of the VCO group. On day 5, the wound bed in all groups was covered by a thin slough. On day 11, the slough was disappeared in the BSS group, while the wound bed in VCO and control group was still covered by slough. On day 14, the wounds in BSS group were almost healed and were covered with new epithelium, while in the VCO group, wounds were still covered with slough. The wound in the control group on day 14 showed no necrotic tissue and the wound bed was filled with granulation tissue.

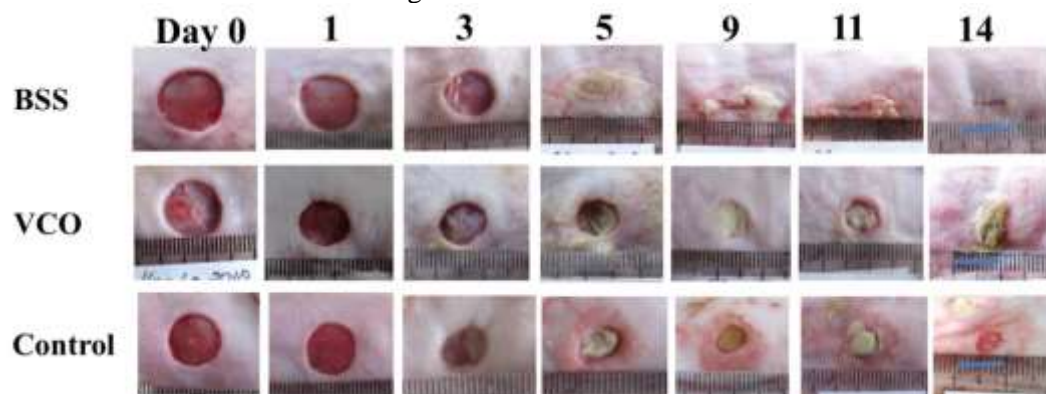


Figure 1. Wound bed appearance

There is no difference in the wound size among the three groups on inflammation phase and early proliferative phase. However, from day 9 onwards, wounds in the BSS group were significantly smaller compared with the ones in the VCO group (day 9, $p=0.034$; day 10, $p=0.034$, day 11, $p=0.34$, day 12, $p=0.034$, day 13, $p=0.034$, and day 14, $p=0.034$) and control group (day 9, $p=0.043$; day 10, $p=0.043$, day 11, $p=0.042$, day 12, $p=0.042$, day 13, $p=0.021$, day 14, $p=0.021$). On day 9, no significant difference was observed between control and VCO group regarding wound size. However, from day 10 onwards, wounds in the control group were significantly smaller compared with the ones

in the VCO group (day 10, $p=0.034$; day 11, $p=0.032$, day 12, $p=0.032$, day 13, $p=0.034$, day 14, $p=0.034$).

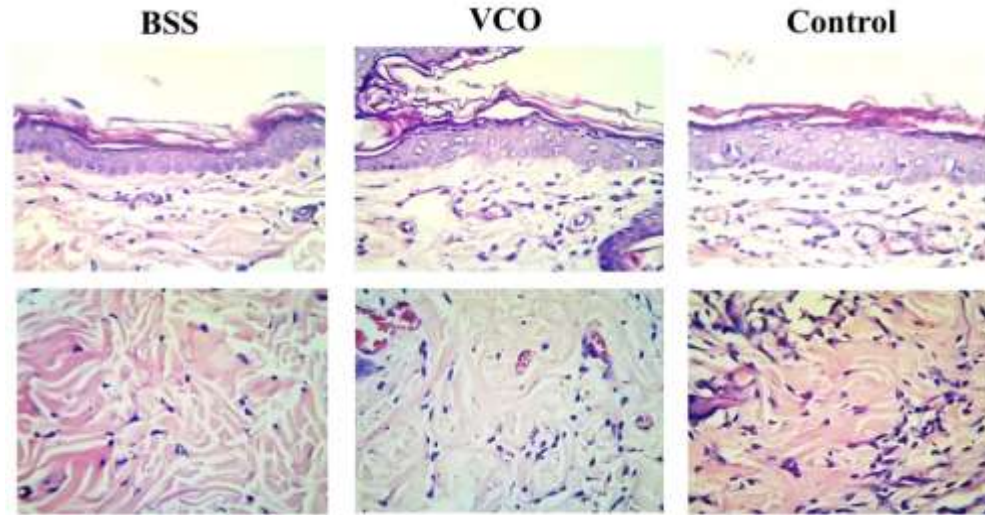


Figure 2. HE staining of epidermis (upper picture) and dermis (lower picture) in the BSS, VCO, and control group on day 4.

3.2. *He staining*

The result of HE staining in epidermis and dermis layer on day 4, and 14 are shown in figure 2, and 3, respectively. Figure 2 shows that the intensity of polymorphonuclear (PMNs) in BSS group was slightly lower than that in control group and VCO groups. The inflammation in the VCO group was also slightly lower than that in control group. Figure 2 also shows that no significant difference was found in the intensity of fibroblast among the three groups on day 4. The result of HE staining on day 14 showed that the intensity of PMNs in BSS group was not only significantly lower compared with control group ($p = 0.008$), but also compared with VCO group ($p = 0.008$). In addition, the level of PMNs in the VCO group was significantly higher than that in the control group ($p = 0.008$). On day 14, the intensity of fibroblast in BSS group was significantly higher compared with control ($p = 0.008$) and VCO group ($p = 0.04$) (Table 1). Figure 3 also shows that the reepithelialization was most advanced in the BSS groups compared with the other groups.

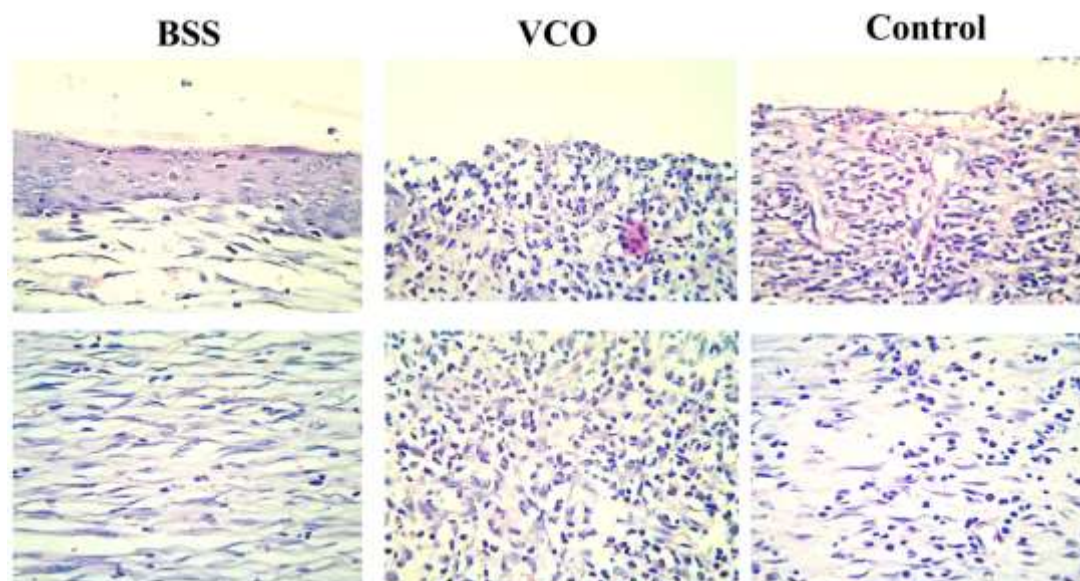


Figure 3. HE staining of epidermis (upper picture) and dermis (lower picture) in the BSS, VCO and control group on day 14.

Table 1. The difference in the intensity of PMNs and Fibroblast in each group on day 14

Groups	PMNs	Fibroblast
BSS	1*†	3*†
VCO	3*	2
Control	2	2

Values represent the median score. Scores can range from 0 to 4 (0 = absent; 1 = occasional; 2 = moderate; 3 = abundant; 4 = very abundant). * $p < 0.05$ (BSS/VCO vs control), † $p < 0.05$ (BSS vs VCO)

4. Discussion

This study is the first comparing BSS and VCO effect in the healing of diabetic ulcers. Our study indicated that wounds treated with BSS healed faster compared with the ones treated with VCO, even compared with the ones treated with use of modern wound-dressing film dressing.

One characteristic of diabetic wounds is a prolonged inflammation due to local hypoxia and bacterial burden. [17] The reduction of inflammation in BSS might be due to the fact that BSS contain anti-inflammatory, antioxidant and antibacterial agents. [4] A recent study investigated ABSS composition, and found that BSS contains antioxidant such as polyphenols, tannin, saponins, and flavonoids. [12] In addition flavonoids have antibacterial activity. Indeed, the extract of banana stem has an antibacterial effect against staphylococcus aureus and pseudomonas aeruginosa. [18]

In this study, the slough in VCO group was thicker than in the other groups. Slough reduction in the BSS and control group might be due to the fact that BSS and film dressing exerted an autolytic debridement, resulting in thinner slough compared with VCO group. In the VCO group, the slough was thicker from day 5 to day 14. The presence of slough causes more extensive inflammation into the surrounding skin, resulting in more tissue damage and accumulation of dead tissue in the wound bed. [17] This might be the reason why the inflammation in the VCO group was higher and more extensive than the other groups on day 14. Our study is not in agreement with a previous study revealing that wounds treated with VCO healed better compared with the ones in the control group. [19] This discrepancy might be due to the fact that in the previous study, the healing ability of a wound treated with VCO was compared with healing ability of an untreated wound, while in this study VCO healing effect was compared with the effect exerted by the modern wound-dressing used in the control group.

Film dressing provides a moist environment in the wound, therefore promoting its reepithelialization.[20]

In this study, BSS also improved the granulation tissue, in agreement with previous studies showing that BSS can improve granulation tissue formation.[21] The formation of granulation tissue is better in BSS than in VCO group and control group. In our study, the level of fibroblast was higher than that in the control and VCO group. Fibroblasts represent the basic materials of collagen (mucopolysaccharides, aminoglysin acid and proline), which is important for granulation tissue formation.[22] A recent study suggested that the mechanism involved in the improvement of the granulation tissue formation may be due to the ability of BSS to increase PDGF-BB.[21] PDGF stimulates fibroblast chemotaxis activity and proliferation, and the production of glycosaminoglycans. Glycosaminoglycans form a gel-like ground substance, in which the collagen fibers are embedded.

This study has one limitation. We investigated the wound healing effect only in term of inflammation, and reepithelialization. However, our study is the first comparing BSS and VCO effect in the healing of diabetic ulcers consecutively and therefore the progression of wound healing was thoroughly observed.

Taken together, our study indicated that BSS was more effective in promoting the healing of diabetic ulcers, and therefore it might be a good candidate in the clinical usage. Nurse and clinician should use BSS over VCO to treat diabetic ulcers. A further study on human subject with diabetic ulcers is needed to confirm these findings.

5. Conclusions

This study compared the effect of BSS, VCO and modern dressing on wound healing of diabetic rats. Our results showed that the inflammation and the presence of slough were the least in BSS group compared with VCO and control group. The reepithelialization was also most advanced in the BSS group. According to our evidences, BSS might be a good candidate for topical treatments of diabetic ulcer.

References

- [1] Shaw J E, Sicree R A and Zimmet P Z 2010 Global estimates of the prevalence of diabetes for 2010 and 2030 *Diabetes Res. Clin. Pract.* **87** 4–14
- [2] Khamaisi M and Balanson S 2017 Dysregulation of wound healing mechanisms in diabetes and the importance of negative pressure wound therapy (NPWT). *Diabetes. Metab. Res. Rev.* **33**
- [3] Haesler E, Watts R, Rice J C K 2016 Local resource botanicals used in wound care *Wound Pr. Res* **24** 84-90.
- [4] Imam M Z and Akter S 2011 Musa paradisiaca L . and Musa sapientum L . : A Phytochemical and Pharmacological Review Mohammad Zafar Imam and Saleha Akter *J. Appl. Pharm. Sci.* **01** 14–20
- [5] SHANMUGAVELU K G and RANGASWAMI G 1962 Tryptophan and Indole Compounds in Banana Ovaries *Nature* **194** 775
- [6] Lewis D A and Shaw G P 2001 A natural flavonoid and synthetic analogues protect the gastric mucosa from aspirin-induced erosions. *J. Nutr. Biochem.* **12** 95–100
- [7] Peumans W J, Zhang W, Barre A, Houles Astoul C, Balint-Kurti P J, Rovira P, Rouge P, May G D, Van Leuven F, Truffa-Bachi P and Van Damme E J 2000 Fruit-specific lectins from banana and plantain. *Planta* **211** 546–54
- [8] Priosoeryanto BP, Putriyanda N, AR L, V J, I W, BF P and R T 2007 The effect of ambon banana stem sap on the acceleration of wound healing process in mice (Mus musculus albinus) *J. Agric Rural Devp* 35–59
- [9] Sachs M, von Eichel J and Asskali F 2002 [Wound management with coconut oil in Indonesian folk medicine]. *Chirurg.* **73** 387–92
- [10] Marina A M, Man Y B C, Nazimah S A H and Amin I 2009 Antioxidant capacity and phenolic

- acids of virgin coconut oil. *Int. J. Food Sci. Nutr.* **60 Suppl 2** 114–23
- [11] Nevin K G and Rajamohan T 2010 Effect of topical application of virgin coconut oil on skin components and antioxidant status during dermal wound healing in young rats. *Skin Pharmacol. Physiol.* **23** 290–7
 - [12] Hendrik Setia Budi, Indah Listiana Kriswandini and A D I 2015 Antioxidant activity test on ambonese banana stem sap (*Musa Dent. J.* **188** 188–92
 - [13] Weremfo A and Adinortey M B 2011 Haemostatic Effect of the Stem Juice of *Musa paradisiaca* L . (*Musaceae*) in Guinea Pigs *Adv. Biol. Res. (Rennes)*. **5** 190–2
 - [14] Sari Y, Hartono, Sutrisna E and Saryono 2019 The effect of short duration of electrical stimulation on wound healing in acute wound in a rat model *Wound Med.* **24** 36–44
 - [15] Sari Y, Purnawan I, Kurniawan D W and Sutrisna E 2018 A Comparative Study of the Effects of *Nigella sativa* Oil Gel and Aloe Vera Gel on Wound Healing in Diabetic Rats. *J. evidence-based Integr. Med.* **23** 2515690X18772804
 - [16] WS R 1997 ImageJ: image processing and analysis in Java
 - [17] Sood A, Granick M S and Tomaselli N L 2014 Wound Dressings and Comparative Effectiveness Data *Adv. wound care* **3** 511–29
 - [18] Amutha K and Selvakumari U 2016 Wound healing activity of methanolic stem extract of *Musa paradisiaca* Linn. (Banana) in Wistar albino rats. *Int. Wound J.* **13** 763–7
 - [19] Soliman A M, Lin T S, Ghafar N A and Das S 2018 Virgin coconut oil and diabetic wound healing : histopathological and biochemical analysis *Eur. J. Anat* **22** 135–44
 - [20] Diegelmann R F and Evans M C 2004 Wound healing: an overview of acute, fibrotic and delayed healing. *Front. Biosci.* **9** 283–9
 - [21] Hs B, Il K and Sa S 2016 Ambonese banana stem sap (*Musa paradisiaca* var . *sapientum*) effect on PDGF-BB expressions and fibroblast proliferation in socket wound healing *Int. J. ChemTech Res.* **9** 558–64
 - [22] Werner S and Grose R 2003 Regulation of wound healing by growth factors and cytokines. *Physiol. Rev.* **83** 835–70