Wound healing activity of skin incision and skin burn from spray gel formula contains combination of banana stem (*Musa acuminata* Colla) and *Aloe vera* ethanol extracts on mice

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Abstract. Various studies reports the activity of wound healing from banana stem (*Musa species*) and also *Aloe vera* individually. This research purpose are to identify the wound healing activity of skin incision and skin burn animal model from spray gel formula contains combination of *Musa paradisiaca* Colla and *Aloe vera* L. ethanol extracts on mice. This research were used randomized post test only control group design. Each of incision wound and burn wound models were used 28 mice and divided into 7 group. Group I was given with banana stem extract (BSE) 10%. Group II was given with *Aloe vera* extract (AVE) 10%. Group III was given with 7.5% BSE and 2.5% AVE. Group IV was given with 5% BSE and 5% AVE. Group V was given with 2.5% BSE and 7.5% AVE. Group VI and group VII (povidone iodine) as vehicle and positive control respectively. All group were sprayed twice a day from first day until seventh day after skin incision/burn induction, and length of wound/wound diameter were observed everyday. The result showed that combination of BSE and AVE had wound healing activity in both wound model which did not different significantly compared with individual extract.

1. Introduction
Wounds are a result of skin injuries that disrupt the other soft tissue. As a respon to injuries, wound healing is a complex process of tissue repair and remodeling [1]. Some research showed that *Musa paradisiaca* stem extract has wound healing activity in burn and incision wound [2,3]. *Aloe vera* also reported has wound healing activity in both wound [4,5]. Some research showed that combination of two or more extracts give a better wound healing activity [6,7]. This research purpose were to identify the wound healing activity of skin incision and skin burn from spray gel formula contains combination of *Musa paradisiaca* Colla stem and *Aloe vera* L ethanol extracts on mice.

2. Experimental
2.1. Material
Fresh *Musa paradisiaca* Colla stem and *Aloe vera* L were obtained from local regency (Sragen, Central Java Indonesia). All formula components were obtained from Pharmaceutical Laboratory Faculty of Mathematics and Natural Sciences Universitas Sebelas Maret Surakarta Central Java Indonesia. All mice were purchased from certified seller in Surakarta regency.
2.2. Preparation and extraction of Musa paradisiaca stem and Aloe vera.
Collected samples were determined in Laboratory Biology of Natural Sciences and Mathematic Faculty Universitas Sebelas Maret. Extraction were made by maseration methods [8].

2.3 Spray gel preparation.
Spray gel of Musa paradisiaca Colla and Aloe vera L ethanol extract and the blended extracts were prepared to the following formula (Table 1). Spray gels were prepared as previously described [9].

Table 1. Spray gel formula.

| Component          | Group I | Group II | Group III | Group IV | Group V | Group VI | Group VII* |
|--------------------|---------|----------|-----------|----------|---------|----------|------------|
| Banana stem extract | 10      | 0        | 7,5       | 5        | 2,5     | -        | -          |
| Aloe vera leaf extract | 0       | 10       | 2,5       | 5        | 7,5     | -        | -          |
| Carbopol 2%         | 10      | 10       | 10        | 10       | 10      | 10       | -          |
| Propyleneglycol     | 2,7     | 2,7      | 2,7       | 2,7      | 2,7     | 2,7      | -          |
| Methyl Paraben      | 0,18    | 0,18     | 0,18      | 0,18     | 0,18    | 0,18     | -          |
| Propyl Paraben      | 0,02    | 0,02     | 0,02      | 0,02     | 0,02    | 0,02     | -          |
| Menthol             | 0,05    | 0,05     | 0,05      | 0,05     | 0,05    | 0,05     | -          |
| Oleum citri         | 4       | 4        | 4         | 4        | 4       | 4        | -          |
| Ethanol 70%         | ad 100  | ad 100   | ad 100    | ad 100   | ad 100  | ad 100   | -          |

*Group VII only contains povidone iodine as spray form

2.4 Experimental protocol.
A total of 56 male Swiss mice weighing between 20-25 g were purchased from local distributor in Surakarta, Central Java, Indonesia. The animals were adapted in caged with 12-h light/12-h dark cycles, controlled temperature, and free access to commercial pellet diet and water ad libitum for a week. All care was taken to minimize the suffering of the animals. The experimental protocol was approved by Health Research Ethics Committee Dr Moewardi General Hospital School of Medicine Universitas Sebelas Maret.

The animals were randomly divided into incision and burn wound groups with each group having 28 animals. Each group was further divided into seven subgroups composed of four animals per subgroup: 1) Banana stem extract (BSE) 10%-treated group, 2) Aloe vera extract (AVE 10%-treated group, 3) blended of BSE and AVE (7,5%:2,5%)-treated group, 4) blended of BSE and AVE (5%:5%)-treated group, 5) blended of BSE and AVE (2,5%:7,5%)-treated group, 6) vehicle group, and 7) Betadine ® (povidone iodine liquid) in spray form as positive group.

2.5 Wound healing activity from skin incision wound.
Animal model of skin incision wound was prepared as previously described [10] with modification. The back area of each animal was shaved approximately 2 cm and cleaned with ethanol 70%. The animals were anesthetized with inhalation of ete. Next, a 1,5 cm long and 0,2 cm depth, midline incision was made through the skin with sharp scalpel. After creating a wound, wound area was cleaned with saline water and sprayed twice a day according to each group for seven days. Healing of incision wounds was evaluated by measuring the length of wound and wound healing percentage for seven days.
Length of wound of each mice was measured three times using digital calipers. Wound healing was calculated using the formula: Wound healing = (length of wound at day 0 – length of wound at day n)/length of wound at day 0, and the result x 100% to get the wound healing percentage (%). Data of wound healing percentage from each group analyzed statistically. A p-value <0.05 was considered statistically significant.

2.6 Wound healing activity from skin incision wound.
Animal model of skin burn wound was prepared as previously described [10] with modification. The back area of each animal was shaved approximately 2 cm and was cleaned with ethanol 70%. The animals were anesthetized with inhalation of ether. Next, a partial thickness burn was made by putting a stainless steel hot plate with diameter 1 cm for 5 seconds on the shaved area. After creating a wound, wound area was cleaned with saline water and sprayed twice a day according to each group for seven days.

Healing of burn wounds was evaluated by measuring the diameter of wound and wound healing percentage for seven days. Diameter of wound of each mice was measured from four different way using digital calipers. Wound healing was calculated using the formula: Wound healing = (diameter of wound at day 0 – diameter of wound at day n)/diameter of wound at day 0, and the result x 100% to get the wound healing percentage (%). Data of wound healing percentage from each group were analyzed statistically. A p-value <0.05 was considered statistically significant.

3. Results and discussions
3.1. Skin incision wound.
Incision wound healing percentage of each group were showed in Table 2.

| Incision Wound Healing Percentage (%) (n=4) |
|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------ |
| Day | Group* | Group* | Group* | Group* | Group* | Group* | Group* |
|-----|--------|--------|--------|--------|--------|--------|--------|
|     | I      | II     | III    | IV     | V      | VI     | VII    |
| 1   | 6.978  | 11.206 | 7.261  | 10.685 | 10.818 | 0.559  | 6.695  |
| 2   | 14.266 | 23.553 | 14.567 | 21.265 | 22.924 | 2.058  | 14.602 |
| 3   | 24.113 | 35.651 | 24.151 | 32.262 | 33.887 | 5.368  | 24.073 |
| 4   | 33.553 | 47.043 | 34.026 | 42.425 | 44.954 | 9.550  | 33.147 |
| 5   | 43.206 | 60.013 | 45.040 | 53.944 | 58.555 | 13.867 | 41.684 |
| 6   | 51.871 | 73.065 | 55.742 | 64.649 | 69.996 | 18.206 | 50.152 |
| 7   | 61.369 | 85.889 | 65.974 | 75.438 | 81.395 | 22.143 | 58.572 |

*p<0.05 compared with negative control from day 1-7

3.2. Skin burn wound
Burn wound healing percentage of each group were showed in Table 3.

| Burn Wound Healing Percentage (%) |
|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| Day | Group* | Group* | Group* | Group* | Group* | Group* | Group* |
|-----|--------|--------|--------|--------|--------|--------|--------|
|     | I      | II     | III    | IV     | V      | VI     | VII    |
| 1   | 8.93   | 13.42  | 9.23   | 9.49   | 8.60   | 2.61   | 4.42   |
| 2   | 16.06  | 23.52  | 18.49  | 20.21  | 20.36  | 7.18   | 7.99   |
| 3   | 20.37  | 29.15  | 20.69  | 25.57  | 26.42  | 10.58  | 14.75  |
| 4   | 24.72  | 34.88  | 25.00  | 30.42  | 31.00  | 13.39  | 19.52  |
| 5   | 29.60  | 40.22  | 29.92  | 34.50  | 34.98  | 15.30  | 23.99  |
| 6   | 33.07  | 44.47  | 33.97  | 38.15  | 38.99  | 16.84  | 28.00  |
| 7   | 36.61  | 50.21  | 37.20  | 41.40  | 43.15  | 18.88  | 31.58  |
Table 2 and Table 3 showed that both of Musa paradisiaca (banana) stem extract (BSE) and Aloe vera extract (AVE) in individual extract and also in blended extract has significant wound healing activity compared with vehicle group (p<0.05). Both of table also showed that wound healing activity from BSE and AVE formulas were not different significantly with individual extract. Nevertheless, Table 2 and Table 3 also showed that Aloe vera extract has higher wound healing percentage compared with Musa paradisiaca extract and blended extracts.

Based on literature review, Aloe vera chemical plant constituents composed of aloins A and B2, aloe resin A, B2, and C. The other important compounds include several sugars (glucose, mannose, cellulose) and various enzymes (oxidase, amylose, catalase), and also vitamins (B1, B2, B6, C, E, folic acid) and minerals (calcium, sodium, magnesium, zinc, copper, and chrome) [11]. The healing property of Aloe vera is related to glucomannan. The glucomannan affects fibroblast growth factor and promotes the activity and proliferation of fibroblasts and in turn induces collagen production and secretion. The mucus of Aloe vera increases amount of collagen and transversal connections among them in wound site. This action accelerates wound improvement [12]. The mucilage of Aloe vera also showed increasing production of fibroelastic growth factors (FGF) and transforming growth factor (TGF) in the wound area [13]. The mucilage also showed accelerates thrombosis, angiogenesis, and better arrangement of collagen on wound site [14]. Aloe vera mucilage also contains vitamins C and E as strong antioxidant and increasing collagen production [15]. The mucilage also possesses antioxidant enzymatic systems such as glutathione peroxidase and superoxide dismutase, which increases wound healing by neutralization the effect of free radicals produced on wound site and with their anti-inflammatory property [16].

Musa paradisiaca stem contain alkaloids, glycosides, saponins, tannins, flavonoids, and reducing sugars [17]. Tannins act as free radical scavenger. Flavonoids accelerates wound healing due to their astringent and antimicrobial activity. Flavonoids also possess potent antioxidant and free radical scavenging effect, and increasing the level of antioxidant enzymes in granuloma tissue. Saponins promote wound healing due to their antioxidant and antimicrobial activity [18].

Wounds usually generates superoxides and lipid peroxidation through neutrophil activation. Therefore any components that inhibits lipid peroxidation is believed to accelerate the viability of collagen fibrils by increasing the strength of collagen fibres, increasing the circulation, preventing the cell damage, and promoting DNA synthesis. Better collagenation seen in wound area under the influence of some herbal extracts may be due to improvement of antioxidant status [19]. Antioxidants can interfere with the oxidation process by reacting with free radicals, chelating catalytic metals, and by acting as oxygen scavenger. Free radicals and other reactive oxygen species (ROSs) are considered to be important causative factors in delaying the healing process [20].

Higher of ROSs concentration can promote severe tissue damage and impede the healing process by causing damage to cellular membranes, DNA, proteins, and lipids. Hence if an herbal extract having antioxidant and antimicrobial property, it could be a good therapeutic agent for increasing the wound healing process [19]. Oxidative stress also plays an important role in wound healing process. This oxidative stress may cause damage to the growing tissue at the repair site[21]. Hence the antioxidants present in the herbal extract could be expected to promote epithelization by controlling oxidative stress. Botanicals with antioxidant or free radical scavenging activity can play significant role in healing of wounds [22].

4. Conclusion
Both of spray gel contain banana stem extract (BSE), Aloe vera extract (AVE), and combination of blended extracts had wound healing activity in incision and burn wound model which did not different significantly compared with individual extract. This research has several limitations including duration of observation which only seven days, and povidon iodine which is used as positive control in both wounds given in spray liquid form. We also do not observed the skin tissue histology and phytochemical screening. Therefore further research need to explore this results.
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References
[1] Thakur R, Jain N, Pathak R and Sandhu S 2011 Evid. Based Complement. and Alternat. Med. 2011 1-17.
[2] Amutha K and Selvakumari U 2016 Int. Wound J. 13 763-67.
[3] Weremfo A, Pappoe A N M and Adinortey M B 2011 RJPPD 6 294-96.
[4] Maenthaisong R, Chaiyakunapruk N, Niruntraporn S and Kongkaew C 2006 Burns 33 713-18.
[5] Yadav K H, Kumar J R and Basha I 2012 Int. J. Pharma. Bio. Sci. 3 63-2.
[6] Aslam M S, Ahmad M S, Mamat A S, Ahmad M Z and Salam F 2016 Evid. Based Complement. and Alternat. Med 2016 1-14.
[7] Elzayat E M, Auda S H, Alanazi F K and Al-Agamy M H 2018 Saudi. Pharm. J. 26 733-38.
[8] Yusuf S, Agunu A and Diana M 2004 J. Ethnopharmacol. 93 33-7.
[9] Iswandana R and Lidya K M S 2017 PSR 4 121-31.
[10] Somboonwong J, Kankaisre M, Tantisira B and Tantisira M H 2012 BMC Complement. Altern. Med. 12 1-7.
[11] Surjushe A, Vasani R and Saple D 2008 Indian J. Dermatol. 53 163-66.
[12] Boudreau M D and Beland F A 2006 J. Environ Sci Health 24 103-54.
[13] Atiba A, Ueno H and Uzuka Y 2011 J. Vet. Med. Sci. 73 583-83.
[14] Oryan A, Naeini A T, Nikahval B and Gorjlan E 2010 Vet Arh 80 509-22.
[15] Kashanian M, Lakeh M M, Ghasemi A and Noori S 2013 J. Reprod. Med 58 34-8.
[16] Hajhashemi V, Ghannadi A and Heidari A H 2012 Res. Pharm. Sci. 7 73-8.
[17] Onyenekwe P C, Okereke O E and Owolewa S O 2013 Curr. Res. J. Biol. Sci. 5 26-9.
[18] Shenoy C, Patil M B, Kumar R and Patil S 2009 Int. J. Pharm. Pharm. Sci. 2 167-75.
[19] Akkol E K, Koca U, Pesin I and Yilmazer D 2011 Evid. Based Complement. and Alternat. Med. 2011 1-7.
[20] Farokhzad O C 2008 Expert. Opin. Drug. Del. 5 927-29.
[21] Wannarat K, Tantisira M H and Tantisira B 2009 Thai J Pharmacol 31 120-3.
[22] Kamath J V, Rana C and Chowdhury A R 2003 Phytother. Res. 17 970-72.