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Wed, Jan 12, 2022 at 1:03 PM

Dear Dr Setia Budi,

Thank you for your recent submission to *Biologics: Targets and Therapy*, titled "Estimation of Platelets Count and Bleeding Time on Peripheral Blood Smear of *Musa paradisiaca* var. *sapientum* (L.) Kuntze Extract" which has been received.

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1 ORIGINAL RESEARCH

2 Hendrik Setia Budi et al

3 **Estimation of Platelets Count and Bleeding Time on**
4 **Peripheral Blood Smear of *Musa paradisiaca* var.**
5 ***sapientum* (L.) Kuntze Extract**

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26 **Abstract:**

27 **Objective:** the aim of this study was to estimate the platelets count and bleeding time on
28 peripheral blood smear of mice tail wound using *Musa paradisiaca* var. *sapientum* (L.) Kuntze
29 (ambonese banana stem extract).

30 **Design:** Randomized post-test only control group design.

31 **Materials and Methods:** Twenty-four male mice (*Mus Musculus*) were randomly divided into 4
32 groups. A negative control group was treated with carboxymethyl cellulose (CMC), a positive
33 control group (K+) treated aspirin 100 mg/kg body weight, group P1 treated with aspirin 100
34 mg/kg body weight and tranexamic acid 50 mg/kg body weight, and group P2 treated with 30% of
35 ambonese banana stem extract (ABSE). The mean and standard deviation data of platelets
36 counts and bleeding time were analyzed by one-way ANOVA statistical software.

37 **Results and Discussion:** The platelets count in the aspirin group was lower than other groups,
38 and obviously there was longer bleeding time. The bleeding time of tranexamic acid and ABSE
39 group was similar, although the platelets count in the ABSE group was lower than in the CMC
40 group.

41 **Conclusion:** This study proved that ambonese banana stem extract has a potency to shorten the
42 bleeding time in mice tail wound without interfering with platelets count.

43 **Keywords:** haemorrhage, health risk, herbal medicine, peripheral blood smears, wound

44

45 **Introduction**

46 Wound is the loss or damage of some body tissues caused by a sharp or blunt trauma,
47 temperature changes, chemicals, burning, electric shock, or animal bites. This damage causes
48 significant morbidity rates worldwide, therefore its management is needed to effectively facilitate
49 the normal healing process for quick recovery.¹⁻³ Open wounds on the body surface cause rupture
50 of blood vessels leading to bleeding. In mild cases, the haemorrhage stops quickly, but when it is
51 wide and deep enough, it takes longer time to stop.⁴ A severe bleeding can cause impaired
52 perfusion, leading to tissue hypoxia which can cause hypovolemic shock.⁵ This condition is due to

53 insufficient blood in the circulation. Therefore, hypoperfusion (low oxygen perfusion) disrupts cell
54 metabolism, and reduces Adenosine Triphosphate (ATP) formation causing general organ
55 failure.⁶

56 Post-extraction teeth haemorrhage can cause severe complications such as uncontrolled
57 bleeding and infection. Wounds after tooth extraction should be well dried because there are
58 various kinds of infectious bacteria in the oral cavity.^{7,8} Blood clotting is a series of complex
59 process to achieve hemostatic state that prevents excessive blood loss after injury.⁹ When
60 bleeding occurs, there are three main processes of hemostasis and coagulation which includes
61 vasoconstriction, platelet aggregation, and activation of blood clotting factors. Therefore, platelets
62 have a major role in the hemostatic phase by forming blood plugs and activating the clotting
63 factors.^{10,11} Furthermore, hemostatic analysis can be performed in the form of peripheral blood
64 smears to ascertain the platelet function through a microscope.^{12,13}

65 However, severe haemorrhage can occur in patients who consume blood-thinning
66 aspirin.¹⁴ Hence, adequate bleeding control can be done by administering systemic hemostatic
67 drugs such as tranexamic acid.¹⁵ Several studies have shown that the administration of
68 hemostatic drug in postoperative patients can have side effects such as nausea, vomiting,
69 headaches, and allergic reactions.¹⁶ In fact, these medications are contraindicated in stroke
70 patients. The use of current coagulant has obvious side effects, hence they have been developed
71 depending on medicinal plants that have minimal side effects.¹⁷

72 There are several plants with medicinal properties which can be used to heal wounds and
73 prevent infections with minimal side effects, therefore they are applied as therapeutic drugs.
74 Besides, it has been found that those plants with active ingredients can play important role in
75 wound healing. In Indonesia, banana trees have been proven contains saponins, flavonoids,
76 alkaloids, tannins and lectins which have various health benefits such as antihypertensive and
77 antidiabetic effects. Furthermore, ambonese banana sap has various therapeutic effects as an
78 antioxidant, antibiotic, and wound healing accelerator.¹⁸ The application of ambonese banana
79 stem sap at a concentration of 30% accelerated wound healing by increasing the expression of
80 Platelet-Derived Growth Factor (PDGF-BB).¹⁹ Effects of PDGF-BB topical treatment in animal

81 models have been shown to improve tissue repair under conditions of delayed wound healing.²⁰
82 Therefore, the aim of this study was to estimate the platelets count and bleeding time on
83 peripheral blood smear of mice tail wound using Ambonese banana stem extract (ABSE) as a
84 medicinal plant in hemostasis.

85 **Material and methods**

86 ***Samples and ethical clearance***

87 This study has passed the ethical clearance of health research no. 586 / HRECC.FODM / IX /
88 2019 Faculty of Dental Medicine, Universitas Airlangga. We used 24 male mice (*Mus Musculus*),
89 weighing between 20-30 g body weight (BW). Sample size formula was obtained from health
90 studies formulation as amount 6 samples each group. In the negative control group (K-), the mice
91 were given carboxymethyl cellulose (CMC) orally. Meanwhile, the positive control group was
92 given 100 mg/kg body weight aspirin dissolved with 1% CMC orally for 7 days. The treatment
93 group-1 (P1) was given aspirin dose of 100 mg/kg body weight dissolved with 1% CMC orally for
94 7 days and given 50 mg/kgBW tranexamic acid orally 30 minutes before cutting the tail. The
95 treatment group-2 (P2) was given aspirin dissolved in 1% CMC orally for 7 days and given ABSE
96 at concentration of 30% 30 minutes before cutting the tail. The solution volume given orally was 1
97 mL/10 g body weight.

98 ***Preparation of ambonese banana stem extract (ABSE)***

99 The stems were obtained from the Plant Conservation Center in Purwodadi Botanical Gardens, at
100 Pasuruan. The plant was identified at LIPI (Indonesian Institute of Sciences) no. 1272 / IPH.06 /
101 HM / XI / 2019, which confirmed it as ambonese banana. The selected plant was 2.5-3 m high
102 with a diameter of around 17-18 cm. Furthermore, the age was between 12-13 months. The stem
103 sap was taken by cutting the bottom and then washing it to remove dirt. Afterwards, they were cut
104 into small pieces with a sterile knife, and distilled water of 200 ml was added till they blend. A
105 homogeneous banana stem sap and aquades were filtered using a bugner funnel connected to a

106 vacuum pump (Gast brand, USA) and given whatman filter paper number 41. The resulted
107 compound was stored in a closed dark bottle to reduce oxidation.²¹

108 ***Platelets count analysis on peripheral blood smears***

109 The last blood drop from the tip of the mice tail was rubbed on a glass object. The platelets count
110 based on the results of peripheral blood smears was then calculated using a counting chamber.
111 The smear was stained with giemsa (Sigma-aldrich, USA) and observed in eight visual fields with
112 a 400x magnification microscope (Nikon H600L, Japan). The blood was placed at a distance of 2-
113 3 mm from the end of the glass. Other smears were made with a different glass and placed at an
114 angle of 30-45 degrees in front of the blood droplets. The smears were left to dry and labeled
115 according to the treatment group. Giemsa dye was diluted with buffer (1:4), then colors were
116 mixed with methanol until it flooded the preparation. The results were then observed in five visual
117 fields under a microscope at 400x magnification.²²

118 ***Tail bleeding time analysis***

119 Bleeding time test was performed on experimental animals. The mice's tail was cleaned with 70%
120 alcohol and cut at a point of 2 cm from the tip using surgical scissors. The dripping blood was
121 absorbed on whatman absorbency paper. Meanwhile, the time from the first blood drip until it
122 stopped was calculated as the bleeding time. Furthermore, the blood was allowed to drip
123 spontaneously and placed on 16 absorbent paper boxes every 15 seconds.²³

124 ***Statistical analysis***

125 The data was analyzed using *Statistical Package for the Social Science Software* (SPSS). It was
126 presented in the form of *mean ± standard deviation* ($\bar{X} \pm SD$). In addition, the normality test of
127 platelets count and bleeding time data used *one sample kolmogorov smirnov*, while the
128 homogeneity used *Levene Test*. The mean difference between groups was analyzed using *one*
129 *way ANOVA* test and *least significant difference (LSD)* at 95% confidence interval.

130 **Results**

131 The data were presented in the form of figures, tables representing mean \pm standard deviation (\bar{X}
132 \pm SD), and graphs. Furthermore, statistical analysis was used to test the hypotheses. The
133 identified stem sap was used according to the previous study.¹⁹

134 ***Phytochemical analysis of ambonese banana stem extract***

135 The active compounds can be qualitatively identified using phytochemical methods.²⁴ Positive
136 results were obtained from the flavonoid, tannin and saponin tests. The flavonoid test was
137 positive because the solution color changed to yellowish-orange. Moreover, the tannin test was
138 positive because there was a color change to dark-green after adding FeCl₃ reagent.
139 Furthermore, the saponin test was positive because there was visible foam formation that was
140 stable for about 10 minutes (Figure 1).

141 ***Histopathological analysis of platelets on peripheral blood***

142 ***smears***

143 The mean of platelets count from 24 mice samples showed 163.3 in CMC group, 78.0 in aspirin
144 group, 169.7 in tranexamic acid group, and 163.0 in ABSE group. Based on these data, the
145 application of tranexamic acid and ABSE increased platelets around the wound compared to the
146 aspirin group, and equal to the CMC group (negative control) (Table 1).

147 According to Table 1, there was a significant difference between groups in platelet count
148 ($p = 0.000$). Tranexamic acid had no significant effect on platelets count compared to CMC group
149 ($p = 0,871$), but administration of aspirin resulted in low platelets count significantly ($p = 0.003$).
150 The platelets count of ABSE and CMC groups were not significant different ($p = 0.937$). The
151 peripheral blood smear with giemsa staining was examined and counted under a light microscopy
152 (400x) (Figure 2).

153 ***Effect of ABSE on the tail bleeding time***

154 The bleeding time of CMC group was 115 secs, and aspirin group was 232.5 secs. It means that
155 bleeding time of aspirin group was longer than negative control group (CMC). Administration of
156 tranexamic acid and ABSE on mice tail wound shorten the bleeding time. In addition, the bleeding
157 time of ABSE group was 95 seconds, it means that it is shorter than that of aspirin group.
158 Therefore, the bleeding time of ABSE was similiar to the bleeding time of tranexamic acid group.
159 It means that ABSE has a great efficacy as a hemostatic agent (Table 2).

160 Based on *One Way ANOVA* test results, a significance value of $p = 0.005$ ($p < 0.01$) was
161 obtained. This means that there were significant differences in bleeding time. Furthermore, there
162 was a marked difference between the CMC and aspirin groups ($p = 0.004$) ($p < 0.01$). Meanwhile,
163 there were significant differences between the aspirin and tranexamic acid groups ($p = 0.004$) (p
164 < 0.01) beside the significant differences between the aspirin and ambonese banana stem extract
165 groups ($p = 0.006$) ($p < 0.01$). However, there was no significant difference between the
166 tranexamic acid and ambonese banana stem extract groups ($p=0.934$). Aspirin has significantly
167 shown prolonged bleeding time than CMC ($p=0.004$), tranexamic acid ($p=0.004$) and ABSE
168 ($p=0.006$), however, there was no difference between the tranexamic acid and ABSE group
169 ($p=0.934$).

170 **Discussion**

171 Ambonese banana stem sap contains tannins, flavonoids, and saponins as antibacterial
172 components. They also stimulate new cell growth in wounds. This sap can accelerate the re-
173 epithelialization process of epidermal tissue, formation of new blood vessels (neocapilerization)
174 and connective tissue (fibroblasts), and the infiltration of inflammatory cells in wounds. The sap
175 active substances that facilitate blood clotting are flavonoids, tannins and saponins.²⁵ Flavonoids
176 and saponins play a vital role in inhibiting the cyclooxygenase cycle by decreasing the production
177 of prostaglandins, which are responsible for vasodilation.²⁶ Tannins can also facilitate platelets

178 adherence to sub-endothelial blood vessels, hence help release adenosine diphosphate (ADP)
179 and thromboxane (TXA₂) mediators.²⁷

180 The active compounds in ambonese banana stem sap have a role in the process of
181 hemostasis which is important to stop bleeding. There are many cells that play a role in this
182 process such as platelets, which are activated by collagen exposure. Therefore, the glycoprotein
183 on the cell surface adheres to the epithelium, which is mediated by von Willebrand Factor (vWF).
184 The adherence to the epithelial surface activates the membranes of alpha granules to release
185 TXA₂ and ADP which are considered major factors in platelets aggregation process. Hence,
186 reducing the bleeding time of wounds or ruptured vessels. After aggregation, a coagulation
187 cascade is initiated which is influenced by extrinsic and intrinsic factors.¹¹ Ambonese banana
188 stem sap contains active compounds that are proven to accelerate wound healing. This is
189 achieved by increasing PDGF-BB which is a growth factor released during platelets aggregation
190 to activate the healing process. Therefore, when PDGF-BB production increases, it shows a high
191 level of aggregation.

192 This research showed longer bleeding time in the aspirin group compared to the CMC,
193 tranexamic acid and ambonese banana stem extract groups. The aspirin group was aimed to
194 prolong the bleeding time. It was reported that administering aspirin for 7 days prolonged
195 bleeding time.²⁸ This drug is a non-steroidal anti-inflammatory drug (NSAID) with blood-thinning
196 property through the inhibition of thromboxane A₂ (TXA₂).²⁹ Therefore, it inhibits blood
197 aggregation and causes prolonged bleeding time.³⁰ The platelets count in the negative control
198 group was lower than CMC, tranexamic acid and ambonese banana stem extract groups.
199 Platelets count decreased in patients on aspirin medication, due to inhibition of aggregation,
200 leading to prolonged bleeding time.

201 The tranexamic acid group have shorter bleeding time, followed by those given the
202 Amboinese banana stem extract. These results are similiar to the previous study which stated
203 that tranexamic acid reduced bleeding time in patients who have been given aspirin.³¹ Meanwhile,
204 there was platelets increase in the tranexamic acid group. This occurred because the compound
205 is an anti-fibrinolytic drug that inhibits the breakdown of fibrin by plasmin through receptors found

206 on plasminogen. These receptors prevents plasmin from binding and finally stabilizes the fibrin
207 matrix.³²

208 Based on the results of the ambonese banana stem extract group, the bleeding time was
209 shorter than the positive control group with an increase in platelets count. Furthermore, a test
210 which stated that the use of 30% ambonese banana stem sap does not show any systemic
211 toxicity on visible tissues in the fibroblasts culture. However, it showed a large number of living
212 fibroblasts accelerated wound healing through the activation of PDGF-BB.¹⁹ The phytochemical
213 test results also showed that the stem sap contained active compounds, including flavonoids,
214 tannins and saponins, which have therapeutic effect in the hemostasis process.

215 The low platelets count in peripheral blood smears compared to the tranexamic acid
216 group is due to inadequate concentrations or dose, hence it affects the potency of the ambonese
217 banana stem sap in hemostasis. The negative control group that was given CMC showed a
218 normal hemostasis. Meanwhile, the positive control group that was given aspirin, tranexamic acid,
219 and amabonese banana stem extract had a longer hemostatic process. The administration of
220 tranexamic acid and ambonese banana stem extract led to a faster coagulation and increased
221 platelets in the wound area, as shown through peripheral blood smears. Based on the discussion
222 above, the use of ambonese banana stem extract in this study gave an effect similar to
223 tranexamic acid as a hemostatic drug. Therefore, it can shorten the bleeding time through
224 platelets activation.

225 **Conclusion**

226 The application of ambonese banana stem extract (*Musa Paradisiaca* Var. *Sapientum* (L) Kuntze)
227 shorten the bleeding time in mice tail wound without interfering to platelets count.

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230 facilities of our experiments.

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233 or not-for-profit sectors.

234 **Disclosure**

235 The authors have declared that there is no conflict of interest.

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Tue, Jun 7, 2022 at 9:55 AM

Dear Dr Budi,

Thank you for your manuscript submission to Journal of Experimental Pharmacology. On behalf of the Editor, I would like to inform you that your submitted manuscript "Estimation of Platelets Count and Bleeding Time on Peripheral Blood Smear of *Musa paradisiaca* var. *sapientum* (L.) Kuntze Extract" (358105) has been peer-reviewed and may be considered for publication after the necessary revisions are completed to the Editors satisfaction.

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Kind Regards,

Soumya Khanapur

On behalf of Professor Paola Rogliani

Editorial Department

Dove Medical Press

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Manuscript ID number:

358105

Title of paper:

Estimation of Platelets Count and Bleeding Time on Peripheral Blood Smear of *Musa paradisiaca* var. *sapientum* (L.) Kuntze Extract

Ethical/Copyright Corrections:

In addition to the approval from the Faculty of Dental Medicine, Universitas Airlangga, please also include the name of the guidelines followed for the welfare of the laboratory animals.

If you have any queries regarding the ethic requirements, please view our Frequently Asked Questions:
https://www.dovepress.com/cr_data/ethics-faq-2019.pdf

Editorial Corrections:

- Author Affiliations: Please update your author affiliations to the following format: department, institution, city, state (if applicable), country
 - Figure legends: Please place the figure legends at the end of the manuscript file.
 - Table Files: Any symbols, italicized or bold/coloured text included within a table must be clarified within the relevant footnotes. Do not include explanations for symbols or fonts that are not used in that specific table. Please correct prior to re-submission.
 - Affiliations/Corresponding Author: The presentation of department/institution details differs between author affiliation list and correspondence details. They both need to align. Kindly check and correct.
 - Your paper has been identified as requiring English language copy editing. Please address this in your revised manuscript to ensure the Editor-in-Chief sees the best possible version of your manuscript when they make their final decision.
-

Response Letter:

A Response to Reviewers letter is required upon resubmission.

We require every comment by the reviewers to be addressed by the authors. Please note, if you feel that some of the reviewer comments will not add value to your manuscript, you do not have to make those particular changes. You may instead respond to the comment in your letter, explaining why you do not agree with or have not made the suggested changes.

Please indicate where in your revised manuscript the changes (as applicable) can be seen. The response letter is used by the Editor to make a decision on whether to accept the manuscript, and so it is vital that every comment is responded to.

Please ensure copies of all figures/tables/supplementary material are provided with the revised manuscript, even if these are not altered during the revisions so we can ensure we have the most up to date file for each.

Reviewer Comments:

Reviewer 1

Title & Abstract

1. Do the title and abstract cover the main aspect of the work?

the title and abstract cover the main aspect of the work

2. Does the introduction provide background and information relevant to the study?

the introduction provide background and information relevant to the study

Material and Methods

3. Are the methods clear and replicable? Do all the results presented match the methods described?

all the results presented match the methods described

Results

4. If relevant are the results novel? Does the study provide an advance in the field? Is the data plausible?

the study provide an advance in the field

Discussion

5. Do the findings described by the author correlate with the results? Are the findings relevant?

the findings described by the author correlate with the results

Conclusion

6. Do the conclusions correlate to the results found?

the conclusions correlate to the results found

Figures & Tables

7. If the author has provided figures and tables are the figures and tables clear and legible? Are the figures free from unnecessary modification?

the author has provided figures and tables are the figures and tables clear and legible

8. Does the paper raise any concerns?

the statistical analysis appropriate to the research

Competing interest

9. Do any of the authors' competing interests raise concerns about the validity of the study i.e. have the authors' competing interests created a bias in the reporting of the results and conclusions?

No

English editing

10. Do you think the manuscript requires English editing to correct the grammar or flow?

Yes

Recommendations to the Editor

Additional comments

1. The abstract should contain typical experimental data to make it persuasive.
 2. For the molecular weight determination, please give the Figure of HPGPC.
 3. The structure of Extract should be determined by 2D NMR.
 4. How is the Extract isolated and purified? Please write down the detailed process, for example: elution curve.
 5. Some references should be added, such as:
2021 Food Chemistry, 361,130089.
2021 Trends in Food Science and Technology, 107, 38-44.
2021 Trends in Food Science and Technology, 109, 211-218.
2021 Food and Function, 12(2), pp. 834-839.
2021 Carbohydrate Polymers, 252,117179.
2021 Trends in Food Science and Technology, 112, pp. 50-57.
2021 Trends in Food Science and Technology, 109, pp. 564-568.
2021 Process Biochemistry, 110, pp. 231-242.
-

Reviewer 2

Title & Abstract

1. Do the title and abstract cover the main aspect of the work?

proper title

2. Does the introduction provide background and information relevant to the study?

The introduction needs to be improved. More information about the plant under investigation needs to be added about its chemistry, folk medicine, and other biological uses.

Material and Methods

3. Are the methods clear and replicable? Do all the results presented match the methods described?

Proper methods are followed. Some minor points need to be corrected:

- Line 107, the word compound needs to be replaced with extract.
- Line 109, what is meant by the last blood drop from the tip of the mice tail?
- Ref. 22 doesn't include the procedure mentioned and it is a very old reference, should be replaced with a more recent reference

Results

4. If relevant are the results novel? Does the study provide an advance in the field? Is the data plausible?

yes

Discussion

5. Do the findings described by the author correlate with the results? Are the findings relevant?

yes, the discussion is well presented

Some minor points need to be cleared:

- Line 171 "Ambonese banana stem sap contains tannins, flavonoids, and saponins as antibacterial components" Add reference.
- Line 180 "The active compounds in ambonese banana stem sap" repeated Line 188, What are these active constituents? should be mentioned in details with suitable references added.
- Line 202 mentioned "Amboinese banana stem extract" while Line 180 mentioned "ambonese banana stem sap ". Is it a sap or extract?? should be cleared.

-

Conclusion

6. Do the conclusions correlate to the results found?

yes, the results are well discussed and proper conclusions are presented but the authors' future recommendations and conclusions need to be added.

Figures & Tables

7. If the author has provided figures and tables are the figures and tables clear and legible? Are the figures free from unnecessary modification?

the figures and tables are clear and legible.

8. Does the paper raise any concerns?

no there are no concerns about these points

Competing interest

9. Do any of the authors' competing interests raise concerns about the validity of the study i.e. have the authors'

competing interests created a bias in the reporting of the results and conclusions?

The authors didn't raise any competing interests or concerns about the validity of the study

English editing

10. Do you think the manuscript requires English editing to correct the grammar or flow?

Yes

Recommendations to the Editor

Additional comments

No additional comments

Reviewer 3

Title & Abstract

1. Do the title and abstract cover the main aspect of the work?

Not completely.

2. Does the introduction provide background and information relevant to the study?

Yes.

Material and Methods

3. Are the methods clear and replicable? Do all the results presented match the methods described?

No, the methods are not stated clearly and will be very difficult to be reproduced, if not properly revised.

Results

4. If relevant are the results novel? Does the study provide an advance in the field? Is the data plausible?

The results seems like validating existing data and previous research works, and struggled with showcasing its novelty.

Discussion

5. Do the findings described by the author correlate with the results? Are the findings relevant?

Yes the findings correlate with the result.

Conclusion

6. Do the conclusions correlate to the results found?

Yes

Figures & Tables

7. If the author has provided figures and tables are the figures and tables clear and legible? Are the figures free from unnecessary modification?

The author needs to provide clear and legible figures, that readily supports the findings.

The table could be edited to avoid misinterpretation of the results presented, (Please see details in latter sections below).

8. Does the paper raise any concerns?

The concerns about this paper is its significance and contribution to the scientific world of knowledge. Also, ethical concerns such as 'cutting' of mice tail, this might draw the attention of animal activists.

Competing interest

9. Do any of the authors' competing interests raise concerns about the validity of the study i.e. have the authors' competing interests created a bias in the reporting of the results and conclusions?

No

English editing

10. Do you think the manuscript requires English editing to correct the grammar or flow?

Yes

Recommendations to the Editor

Additional comments

-A very thorough language editing is strongly recommended.

-The authors should highlight the significance of this study as many articles have been published on the plant, this methods and the models, the route used in this study. What did the authors do differently from similar research works?

-the scientific name of the plant should be put in italics throughout the text.

Title:

This title is somewhat inappropriate and truncated, it should be concise, succinct and reflect 'the what', 'the how' and 'the where' it was done such that it is structured like the below for an example;

"Estimation of Platelets count and Bleeding time of mice treated with *Musa paradisiaca* extract"

Abstract:

-The objective should be rephrased...bleeding time cannot be estimated using peripheral blood smear.

- not sure the word "obviously" in the 'Result and Discussion' was correctly used, rather say "...and there was a significantly longer bleeding time."

Introduction:

-the first sentence in paragraph 2 lacks connectivity with the preceding one, pls find a meeting point or insert somewhere more appropriate.

-kindly rephrase the last sentence of paragraph 2. Please see the below example, it doesn't have to be stated exactly like that but the points should be showcased, understandable and clear to readers: "Hence, the significance of hemostatic analysis to estimate platelet function, which could be done on a peripheral blood smear under a light microscope".

-advisable to rephrase last sentence of paragraph 3

Materials and Methods:

-it is advisable to replace the word 'cutting the tail' to avoid animal ethical issues. words such as 'nipping the tail' may be used.

-State the significance of CMC, for example the mice were given CMC orally to induce or as treatment or standard drug.

-"other smear"...Do you mean other technical samples? And if yes, why use a different type of glass?

-It was mentioned earlier in this section that 8 visual fields were observed, why 5 now?

-In the collection of the plant material used, was the sap allowed to solidify before it was cut in pieces with knife, kindly state clearly.

-Note, if the steps outlined in the section 'Platelet count analysis on peripheral blood smears' described just one type of slide preparation, then they should be aligned chronological, as they were carried out.

For instance, platelet count can't be done before staining and allowing the slide to dry, whereas it was stated in the text, in that order.

-"The last blood drop from the tip of the mice tail"....can these be stated more explicitly? How is the last drop of blood from the mice tail collected? Is the mice dead?

Results:

-"The identified stem sap was used according to the previous study"....This should be removed and included under "Materials and Methods"

-"The active compounds can be qualitatively identified using phytochemical methods"....This should also be removed and placed in the Methods and Materials, with a summary of the methods employed.

-Not sure 'histopathological analysis' is most appropriate word, histopathology studies the anatomy and any change in the physical structure of the cells/tissues. There were no histopathology of platelet reported here, only platelet count.

Discussion:

-All the treatment groups ought to be given aspirin, to be able to compare the effect of the interventions (extract and standard drug) in the treated groups against the control group given aspirin only.

Figures and Tables

Figure 2:

-The authors need to resubmit clearer plates that distinctively show platelets which is the cells of interest in this study. For example, the black arrows which are meant to point out platelets are pointing to red blood cells. A more detailed plate/picture should be provided.

Tables 1 and 2:

-it might not be necessary to include the 'p-value' column/field on the table, as the information on the table makes sense without the p-value column. It can be confusing to readers that 2 different p-values are presented here for the same data and analysis.

Your revised files have been successfully submitted [358105]

Soumya Khanapur <soumyakhanapur@dovepress.com>
Reply-To: Soumya Khanapur <soumyakhanapur@dovepress.com>
To: Dr Budi <hendrik-s-b@fkg.unair.ac.id>

Wed, Jun 15, 2022 at 7:21 PM

Dear Dr Budi,

Thank you for submitting your revised manuscript and additional files to Journal of Experimental Pharmacology. These have been uploaded successfully. We will begin processing the submission in the next few days, and will be in contact with an update once we have performed our Editorial Checks.

We have received the following files:

1. 15_Jun_2022_manuscript-revision.docx
2. 15_Jun_2022_manuscript-revision_1655294234.docx
3. 15_Jun_2022_Figure_1.jpg
4. 15_Jun_2022_Figure_2.jpg
5. 15_Jun_2022_Table_1.docx
6. 15_Jun_2022_Table_2.docx
7. 16_Jun_2022_Graphical_Abstract.jpg
8. 15_Jun_2022_Resubmission_statement.docx
9. 15_Jun_2022_Response_to_reviewer.docx

If you have any queries, or if there is a problem with any of the uploaded files, please email the revised manuscript co-ordinator below and they can help get it corrected.

Regards,
Soumya Khanapur
Revised Manuscript Co-ordinator
soumyakhanapur@dovepress.com
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358105

Dear Editor

Professor Paola Rogliani

Thank you for giving us the opportunity to submit a revised draft of the manuscript “Estimation of Platelets Count and Bleeding Time of Mice Treated with *Musa paradisiaca* var. *sapientum* (L.) Kuntze Extract” for publication in the Journal of Experimental Pharmacology.

We appreciate the time and effort that you and the reviewers dedicated to providing feedback on our manuscript and are grateful for the insightful comments and valuable improvements to our paper. We have incorporated most of the suggestions made by the reviewers. Those changes are highlighted within the manuscript. Please see in the attachment file, in blue, for a

Thank you very much for your kindness.

Best Regards

Assoc. Prof. Dr. Hendrik Setia Budi, DDS., MDS

Response to Reviewers' comments

Editor:	Response:
<p>Ethical/Copyright Corrections: In addition to the approval from the Faculty of Dental Medicine, Universitas Airlangga, please also include the name of the guidelines followed for the welfare of the laboratory animals.</p>	<p>Thank you for your feedback and review. We have revised it in the manuscript.</p>
<p>Editorial Corrections:</p> <ul style="list-style-type: none"> • Author Affiliations: Please update your author affiliations to the following format: department, institution, city, state (if applicable), country • Figure legends: Please place the figure legends at the end of the manuscript file. • Table Files: Any symbols, italicized or bold/coloured text included within a table must be clarified within the relevant footnotes. Do not include explanations for symbols or fonts that are not used in that specific table. Please correct prior to resubmission. • Affiliations/Corresponding Author: The presentation of department/institution details differs between author affiliation list and correspondence details. They both need to align. Kindly check and correct. • Your paper has been identified as requiring English language copy editing. Please address this in your revised manuscript to ensure the Editor-in-Chief sees the best possible version of your manuscript when they make their final decision 	<p>Thank you for your feedback and suggestion. We have revised it in the manuscript.</p> <p>Thank you for your feedback and review. We have added it in the manuscript.</p> <p>Thank you for your feedback and review. We have declared that only relevant information which explain in the legends. The revision has added it in the manuscript carefully.</p> <p>Thank you for your feedback and review. We have revised it in the manuscript carefully.</p> <p>Thank you for your feedback, that we have sent the manuscript for English editing before submit it to the journal. The proof of language editing enclosed in this attachment files.</p>

Reviewer-1	Response
<p>Comments-1: The abstract should contain typical experimental data to make it persuasive.</p>	<p>Thank you for your feedback and review. We have revised it in the manuscript carefully.</p>
<p>Comments-2: For the molecular weight determination, please give the Figure of HPGPC.</p>	<p>Thank you for your feedback and review. The HPGPC was included in the other paper and still in review.</p>
<p>Comments-3: The structure of Extract should be determined by 2D NMR.</p>	<p>Thank you for your feedback and review. We didn't determine the extract's structure for NMR analysis.</p>
<p>Comments-4: How is the Extract isolated and purified? Please write down the detailed process, for example: elution curve.</p>	<p>Thank you for your feedback and review. We used the crude extract of this plant with maceration. The extract and solvent were separated by a rotary evaporator (Heidolp) at a temperature 50 °C and a speed of 200 rpm for 2 h until a condensed extract was obtained.</p>
<p>Comments-5: Some references should be added, such as: 2021 Food Chemistry, 361,130089. 2021 Trends in Food Science and Technology, 107, 38-44. 2021 Trends in Food Science and Technology, 109, 211-218. 2021 Food and Function, 12(2), pp. 834-839. 2021 Carbohydrate Polymers, 252,117179. 2021 Trends in Food Science and Technology, 112, pp. 50-57. 2021 Trends in Food Science and Technology, 109, pp. 564-568. 2021 Process Biochemistry, 110, pp. 231-242.</p>	<p>Thank you for your feedback, we have added one of them to my references in the manuscript.</p>

Reviewer-2	Response
<p>Comment-1: The introduction needs to be improved. More information about the plant under investigation needs to be added about its chemistry, folk medicine, and other biological uses.</p>	<p>Thank you for your feedback and review. We have provided the active compounds and herb medical used of previous study in the last paragraphs of introduction.</p>
<p>Comments-2: Proper methods are followed. Some minor points need to be corrected:</p> <ul style="list-style-type: none"> - Line 107, the word compound needs to be replaced with extract. - Line 109, what is meant by the last blood drop from the tip of the mice tail? - Ref. 22 doesn't include the procedure mentioned and it is a very old reference, should be replaced with a more recent reference. 	<p>Thank you for your feedback and review. We have revised it in the manuscript carefully.</p> <p>Thank you for your feedback and review. We have revised it in the manuscript and provide a sentence for detail. Blood samples are taken through a scalpel cut near the tail tip. The last drop of blood was rubbed on a glass object.</p> <p>We have changed to the new reference on reference number 23.</p>
<p>Comments-3: yes, the discussion is well presented Some minor points need to be cleared:</p> <ul style="list-style-type: none"> - Line 171 "Ambonese banana stem sap contains tannins, flavonoids, and saponins as antibacterial components" Add reference. - Line 180 "The active compounds in ambonese banana stem sap" repeated Line 188, What are these active constituents? should be mentioned in details with suitable references added. - Line 202 mentioned "Amboinese banana stem extract" while Line 180 mentioned "ambonese banana stem sap ". Is it a sap or extract?? should be cleared. 	<p>Thank you for your feedback and review. We have added the reference for this statement.</p> <p>Thank you for your feedback and review. In the previous statement on discussion section, we have provide the active compound in this plant and reference too.</p> <p>Thank you for your feedback and review. According to our previous study, the active compounds have found in sap. We used extracted methods to get more sap from stem of this plant.</p>
<p>Comments-4: yes, the results are well discussed and proper conclusions are presented but the authors' future recommendations and conclusions need to be added</p>	<p>Thank you for your feedback and review. We have added the recommendation for the future in the conclusion.</p>

Reviewer-3	Response
<p>Comment-1: The authors should highlight the significance of this study as many articles have been published on the plant, this methods and the models, the route used in this study. What did the authors do differently from similar research works?</p>	<p>Thank you for your feedback and review. <i>Musa paradisiaca</i> is tropical plant in Indonesia, and have some properties as folk medicine in previous study. As an accelerating wound healing, this plant has a potential to PDGF-BB expression to improve the platelet's function. Based on this study, we want to know the effect to coagulation specifically.</p>
<p>Comments-2: The scientific name of the plant should be put in italics throughout the text.</p>	<p>Thank you for your feedback and review. We have revised it in the manuscript carefully.</p>
<p>Comments-3: Title: This title is somewhat inappropriate and truncated, it should be concise, succinct and reflect 'the what', 'the how' and 'the where' it was done such that it is structured like the below for an example; "Estimation of Platelets count and Bleeding time of mice treated with <i>Musa paradisiaca</i> extract"</p>	<p>Thank you for your feedback and review. We have revised it in the manuscript carefully.</p>
<p>Comments-4: Abstract: -The objective should be rephrased...bleeding time cannot be estimated using peripheral blood smear. - not sure the word "obviously" in the 'Result and Discussion' was correctly used, rather say "...and there was a significantly longer bleeding time."</p>	<p>Thank you for your feedback and review. We measured peripheral blood smear to provide platelets count, but bleeding time is measured on blood samples through a scalpel cut near the tail tip. Time from the first blood drip until it stopped was calculated as the bleeding time.</p>
<p>Comments-5: Introduction: -the first sentence in paragraph 2 lacks connectivity with the preceding one, pls find a meeting point or insert somewhere more appropriate. -kindly rephrase the last sentence of paragraph 2. Please see the below example, it doesn't have to be stated exactly like that but the points should be showcased, understandable and clear to readers: "Hence, the significance of</p>	<p>Thank you for your feedback and review. Actually, we have written wound healing and bleeding/haemorrhage phenomena as common in the first paragraph. We make relation between first and second paragraph with statement on post-extraction teeth haemorrhage can cause severe complications such as uncontrolled bleeding and infection.</p> <p>Thank you for your feedback and review. we have revised it in the manuscript carefully.</p>

<p>hemostatic analysis to estimate platelet function, which could be done on a peripheral blood smear under a light microscope".</p> <p>-advisable to rephrase last sentence of paragraph 3</p>	<p>Thank you for your feedback and review. We have revised it in the manuscript carefully.</p>
<p>Comments-6: Materials and Methods:</p> <p>-it is advisable to replace the word 'cutting the tail' to avoid animal ethical issues. words such as 'nipping the tail' may be used.</p> <p>-State the significance of CMC, for example the mice were given CMC orally to induce or as treatment or standard drug.</p> <p>-"other smear"...Do you mean other technical samples? And if yes, why use a different type of glass?</p> <p>-It was mentioned earlier in this section that 8 visual fields were observed, why 5 now?</p> <p>-In the collection of the plant material used, was the sap allowed to solidify before it was cut in pieces with knife, kindly state clearly.</p> <p>-Note, if the steps outlined in the section 'Platelet count analysis on peripheral blood smears' described just one type of slide preparation, then they should be aligned chronological, as they were carried out. For instance, platelet count can't be done before staining and allowing the slide to dry, whereas it was stated in the text, in that order.</p>	<p>Thank you for your feedback and review. according the previous study, that cutting the tail is a standard method to prove the bleeding time. This study has provided an ethical clearance in compliance with Government Regulation of the Republic of Indonesia Number 95 of 2012 Concerning Veterinary Public Health and Animal Welfare.</p> <p>Thank you for your feedback and review. CMC is one of the cellulose derivatives that is used the most frequently. CMC has been utilized as an excipient in a variety of sectors, including those associated with food, pharmaceuticals, and cosmetics, which has no effect to the material combination or cells.</p> <p>Thank you for your feedback and review. It mean that one glass object for one sample. If we have more samples, so we should use the other glass.</p> <p>Thank you for your feedback and review. The correct visual field are five. We have revised it in the manuscript.</p> <p>Thank you for your feedback and review. According to the information included in the manuscript, the stem was cut into small pieces using a clean knife, and then 200 ml of distillation water was added in order to obtain the required quantity of sap.</p> <p>Thank you for your feedback and review. Yes, we agree with you. The smears were left to dry and labeled of each group. Giemsa dye was diluted with buffer (1:4), then colors were mixed with methanol until it flooded the preparation.</p>

<p>- "The last blood drop from the tip of the mice tail"....can these be stated more explicitly? How is the last drop of blood from the mice tail collected? Is the mice dead?</p>	<p>Thank you for your feedback and review. The mice is not die, but the blood was allowed to drip spontaneously to absorbent paper boxes every 15 seconds. We have times between 1-15 second to know that is the last bleeding, and put the last blood drop into the glass object.</p>
<p>Comments-7: Results: - "The identified stem sap was used according to the previous study"....This should be removed and included under "Materials and Methods" - "The active compounds can be qualitatively identified using phytochemical methods"....This should also be removed and placed in the Methods and Materials, with a summary of the methods employed. - Not sure 'histopathological analysis' is most appropriate word, histopathology studies the anatomy and any change in the physical structure of the cells/tissues. There were no histopathology of platelet reported here, only platelet count.</p>	<p>Thank you for your feedback and review. We have revised it in the manuscript carefully. Thank you for your feedback and review. We have revised it in the manuscript carefully. Thank you for your feedback and review. I have removed histopathology in the text if it is not appropriate. Histopathology is the diagnosis and study of diseases of the tissues, and involves examining tissues and/or cells under a microscope. Platelet is a cell that examining under microscope.</p>
<p>Comments-8: Discussion: - All the treatment groups ought to be given aspirin, to be able to compare the effect of the interventions (extract and standard drug) in the treated groups against the control group given aspirin only.</p>	<p>Thank you for your feedback and review. Yes, aspirin was administered to all groups for 7 days, with the exception of the control group in order to increase the bleeding time. Extract and tranexamid acid were administered to the treatment groups only.</p>
<p>Comments-9: Figures and Tables Figure 2: - The authors need to resubmit clearer plates that distinctively show platelets which is the cells of interest in this study. For example, the black arrows which are meant to point out platelets are pointing to red blood cells. A more detailed plate/picture should be provided.</p>	<p>Thank you for your feedback and review. We have submitted the new figure accurately reflect.</p>
<p>Comments-10: Tables 1 and 2: - it might not be necessary to include the 'p-value' column/field on the table, as the information on the table makes sense without</p>	<p>Thank you for your feedback and review. We have revised it in the manuscript carefully.</p>

<p>the p-value column. It can be confusing to readers that 2 different p-values are presented here for the same data and analysis</p>	
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Response to Reviewers' comments

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<p>Ethical/Copyright Corrections: In addition to the approval from the Faculty of Dental Medicine, Universitas Airlangga, please also include the name of the guidelines followed for the welfare of the laboratory animals.</p>	<p>Thank you for your feedback and review. We have revised it in the manuscript.</p>
<p>Editorial Corrections:</p> <ul style="list-style-type: none"> • Author Affiliations: Please update your author affiliations to the following format: department, institution, city, state (if applicable), country • Figure legends: Please place the figure legends at the end of the manuscript file. • Table Files: Any symbols, italicized or bold/coloured text included within a table must be clarified within the relevant footnotes. Do not include explanations for symbols or fonts that are not used in that specific table. Please correct prior to resubmission. • Affiliations/Corresponding Author: The presentation of department/institution details differs between author affiliation list and correspondence details. They both need to align. Kindly check and correct. • Your paper has been identified as requiring English language copy editing. Please address this in your revised manuscript to ensure the Editor-in-Chief sees the best possible version of your manuscript when they make their final decision 	<p>Thank you for your feedback and suggestion. We have revised it in the manuscript.</p> <p>Thank you for your feedback and review. We have added it in the manuscript.</p> <p>Thank you for your feedback and review. We have declared that only relevant information which explain in the legends. The revision has added it in the manuscript carefully.</p> <p>Thank you for your feedback and review. We have revised it in the manuscript carefully.</p> <p>Thank you for your suggest, that we have sent the manuscript for English editing before submit to the journal.</p>

Reviewer-1	Response
<p>Comments-1: The abstract should contain typical experimental data to make it persuasive.</p>	<p>Thank you for your feedback and review. We have revised it in the manuscript carefully.</p>
<p>Comments-2: For the molecular weight determination, please give the Figure of HPGPC.</p>	<p>Thank you for your feedback and review. The HPGPC was included in the other paper and still in review.</p>
<p>Comments-3: The structure of Extract should be determined by 2D NMR.</p>	<p>Thank you for your feedback and review. We didn't determine the extract's structure for NMR analysis.</p>
<p>Comments-4: How is the Extract isolated and purified? Please write down the detailed process, for example: elution curve.</p>	<p>Thank you for your feedback and review. We used the crude extract of this plant with maceration. The extract and solvent were separated by a rotary evaporator (Heidolp) at a temperature 50 °C and a speed of 200 rpm for 2 h until a condensed extract was obtained.</p>
<p>Comments-5: Some references should be added, such as: 2021 Food Chemistry, 361,130089. 2021 Trends in Food Science and Technology, 107, 38-44. 2021 Trends in Food Science and Technology, 109, 211-218. 2021 Food and Function, 12(2), pp. 834-839. 2021 Carbohydrate Polymers, 252,117179. 2021 Trends in Food Science and Technology, 112, pp. 50-57. 2021 Trends in Food Science and Technology, 109, pp. 564-568. 2021 Process Biochemistry, 110, pp. 231-242.</p>	<p>Thank you for your feedback, we have added one of them to my references in the manuscript.</p>

Reviewer-2	Response
<p>Comment-1: The introduction needs to be improved. More information about the plant under investigation needs to be added about its chemistry, folk medicine, and other biological uses.</p>	<p>Thank you for your feedback and review. We have provided the active compounds and herb medical used of previous study in the last paragraphs of introduction.</p>
<p>Comments-2: Proper methods are followed. Some minor points need to be corrected: - Line 107, the word compound needs to be replaced with extract. - Line 109, what is meant by the last blood drop from the tip of the mice tail? - Ref. 22 doesn't include the procedure mentioned and it is a very old reference, should be replaced with a more recent reference.</p>	<p>Thank you for your feedback and review. We have revised it in the manuscript carefully.</p> <p>Thank you for your feedback and review. We have revised it in the manuscript and provide a sentence for detail. Blood samples are taken through a scalpel cut near the tail tip. The last drop of blood was rubbed on a glass object.</p> <p>We have changed to the new reference on reference number 23.</p>
<p>Comments-3: yes, the discussion is well presented Some minor points need to be cleared: - Line 171 "Ambonese banana stem sap contains tannins, flavonoids, and saponins as antibacterial components" Add reference. - Line 180 "The active compounds in ambonese banana stem sap" repeated Line 188, What are these active constituents? should be mentioned in details with suitable references added.</p>	<p>Thank you for your feedback and review. We have added the reference for this statement.</p> <p>Thank you for your feedback and review. In the previous statement on discussion section, we have provide the active compound in this plant and reference too.</p>

<p>- Line 202 mentioned "Amboinese banana stem extract" while Line 180 mentioned "ambonese banana stem sap ". Is it a sap or extract?? should be cleared.</p>	<p>Thank you for your feedback and review. According to our previous study, the active compounds have found in sap. We used extracted methods to get more sap from stem of this plant.</p>
<p>Comments-4: yes, the results are well discussed and proper conclusions are presented but the authors' future recommendations and conclusions need to be added</p>	<p>Thank you for your feedback and review. We have added the recommendation for the future in the conclusion.</p>

Reviewer-3	Response
<p>Comment-1: The authors should highlight the significance of this study as many articles have been published on the plant, this methods and the models, the route used in this study. What did the authors do differently from similar research works?</p>	<p>Thank you for your feedback and review. <i>Musa paradisiaca</i> is tropical plant in Indonesia, and have some properties as folk medicine in previous study. As an accelerating wound healing, this plant has a potential to PDGF-BB expression to improve the platelet's function. Based on this study, we want to know the effect to coagulation specifically.</p>
<p>Comments-2: The scientific name of the plant should be put in italics throughout the text.</p>	<p>Thank you for your feedback and review. We have revised it in the manuscript carefully.</p>
<p>Comments-3: Title: This title is somewhat inappropriate and truncated, it should be concise, succinct and reflect 'the what', 'the how' and 'the where' it was done such that it is structured like the below for an example; "Estimation of Platelets count and Bleeding time of mice treated with <i>Musa paradisiaca</i> extract"</p>	<p>Thank you for your feedback and review. We have revised it in the manuscript carefully.</p>
<p>Comments-4: Abstract: -The objective should be rephrased...bleeding time cannot be estimated using peripheral blood smear. - not sure the word "obviously" in the 'Result and Discussion' was correctly used, rather say "...and there was a significantly longer bleeding time."</p>	<p>Thank you for your feedback and review. We measured peripheral blood smear to provide platelets count, but bleeding time is measured on blood samples through a scalpel cut near the tail tip. Time from the first blood drip until it stopped was calculated as the bleeding time.</p>
<p>Comments-5: Introduction: -the first sentence in paragraph 2 lacks connectivity with the preceding one, pls find</p>	<p>Thank you for your feedback and review. Actually, we have written wound healing and bleeding/haemorrhage phenomena as</p>

<p>a meeting point or insert somewhere more appropriate.</p> <p>-kindly rephrase the last sentence of paragraph 2. Please see the below example, it doesn't have to be stated exactly like that but the points should be showcased, understandable and clear to readers: "Hence, the significance of hemostatic analysis to estimate platelet function, which could be done on a peripheral blood smear under a light microscope".</p> <p>-advisable to rephrase last sentence of paragraph 3</p>	<p>common in the first paragraph. We make relation between first and second paragraph with statement on post-extraction teeth haemorrhage can cause severe complications such as uncontrolled bleeding and infection.</p> <p>Thank you for your feedback and review. we have revised it in the manuscript carefully.</p> <p>Thank you for your feedback and review. We have revised it in the manuscript carefully.</p>
<p>Comments-6: Materials and Methods:</p> <p>-it is advisable to replace the word 'cutting the tail' to avoid animal ethical issues. words such as 'nipping the tail' may be used.</p> <p>-State the significance of CMC, for example the mice were given CMC orally to induce or as treatment or standard drug.</p> <p>-"other smear"...Do you mean other technical samples? And if yes, why use a different type of glass?</p> <p>-It was mentioned earlier in this section that 8 visual fields were observed, why 5 now?</p>	<p>Thank you for your feedback and review. according the previous study, that cutting the tail is a standard method to prove the bleeding time. This study has provided an ethical clearance in compliance with Government Regulation of the Republic of Indonesia Number 95 of 2012 Concerning Veterinary Public Health and Animal Welfare.</p> <p>Thank you for your feedback and review. CMC is one of the cellulose derivatives that is used the most frequently. CMC has been utilized as an excipient in a variety of sectors, including those associated with food, pharmaceuticals, and cosmetics, which has no effect to the material combination or cells.</p> <p>Thank you for your feedback and review. It mean that one glass object for one sample. If we have more samples, so we should use the other glass.</p> <p>Thank you for your feedback and review. The correct visual field are five. We have revised it in the manuscript.</p> <p>Thank you for your feedback and review. According to the information included in the</p>

<p>-In the collection of the plant material used, was the sap allowed to solidify before it was cut in pieces with knife, kindly state clearly.</p> <p>-Note, if the steps outlined in the section 'Platelet count analysis on peripheral blood smears' described just one type of slide preparation, then they should be aligned chronological, as they were carried out. For instance, platelet count can't be done before staining and allowing the slide to dry, whereas it was stated in the text, in that order.</p> <p>-"The last blood drop from the tip of the mice tail"....can these be stated more explicitly? How is the last drop of blood from the mice tail collected? Is the mice dead?</p>	<p>manuscript, the stem was cut into small pieces using a clean knife, and then 200 ml of distillation water was added in order to obtain the required quantity of sap.</p> <p>Thank you for your feedback and review. Yes, we agree with you. The smears were left to dry and labeled of each group. Giemsa dye was diluted with buffer (1:4), then colors were mixed with methanol until it flooded the preparation.</p> <p>Thank you for your feedback and review. The mice is not die, but the blood was allowed to drip spontaneously to absorbent paper boxes every 15 seconds. We have times between 1-15 second to know that is the last bleeding, and put the last blood drop into the glass object.</p>
<p>Comments-7: Results: -"The identified stem sap was used according to the previous study"....This should be removed and included under "Materials and Methods"</p> <p>-"The active compounds can be qualitatively identified using phytochemical methods"....This should also be removed and placed in the Methods and Materials, with a summary of the methods employed.</p> <p>-Not sure 'histopathological analysis' is most appropriate word, histopathology studies the anatomy and any change in the physical structure of the cells/tissues. There were no histopathology of platelet reported here, only platelet count.</p>	<p>Thank you for your feedback and review. We have revised it in the manuscript carefully.</p> <p>Thank you for your feedback and review. We have revised it in the manuscript carefully.</p> <p>Thank you for your feedback and review. I have removed histopathology in the text if it is not appropriate. Histopathology is the diagnosis and study of diseases of the tissues, and involves examining tissues and/or cells under a microscope. Platelet is a cell that examining under microscope.</p>
<p>Comments-8: Discussion:</p>	<p>Thank you for your feedback and review. Yes, aspirin was administered to all groups for 7 days, with the exception of the control</p>

<p>-All the treatment groups ought to be given aspirin, to be able to compare the effect of the interventions (extract and standard drug) in the treated groups against the control group given aspirin only.</p>	<p>group in order to increase the bleeding time. Extract and tranexamid acid were administered to the treatment groups only.</p>
<p>Comments-9: Figures and Tables Figure 2: -The authors need to resubmit clearer plates that distinctively show platelets which is the cells of interest in this study. For example, the black arrows which are meant to point out platelets are pointing to red blood cells. A more detailed plate/picture should be provided.</p>	<p>Thank you for your feedback and review. We have submitted the new figure accurately reflect.</p>
<p>Comments-10: Tables 1 and 2: -it might not be necessary to include the 'p-value' column/field on the table, as the information on the table makes sense without the p-value column. It can be confusing to readers that 2 different p-values are presented here for the same data and analysis</p>	<p>Thank you for your feedback and review. We have revised it in the manuscript carefully.</p>

Journal of Experimental Pharmacology – Revised Manuscript Corrections

Soumya Khanapur <soumyakhanapur@dovepress.com>
Reply-To: Soumya Khanapur <soumyakhanapur@dovepress.com>
To: Dr Budi <hendrik-s-b@fkg.unair.ac.id>

Thu, Jun 30, 2022 at 9:08 AM

Dear Dr Budi

I am just contacting you regarding your revised manuscript which was recently resubmitted titled 'Estimation of Platelets Count and Bleeding Time on Peripheral Blood Smear of *Musa paradisiaca* var. *sapientum* (L.) Kuntze Extract' (ID: 358105)

Upon beginning to process your revised manuscript, we have noticed some issues that prevent us from forwarding your submission to the Editor for final editorial review. Please use the link below to find a list of your files which need further correction. All the required corrections can be found in the included manuscript file as comments.

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Once we receive the corrected files, we will continue processing your submission, and provided we do not encounter any further issues which need correcting, will notify you once it has been sent to the Editor for final decision.

Please don't hesitate to contact me if you have queries about any of the requested corrections.

Regards,
Soumya Khanapur
Revised Manuscript Co-ordinator
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Soumya Khanapur <soumyakhanapur@dovepress.com>
Reply-To: Soumya Khanapur <soumyakhanapur@dovepress.com>
To: Dr Budi <hendrik-s-b@fkg.unair.ac.id>

Thu, Jun 30, 2022 at 5:28 PM

Dear Dr Budi,

Thank you for submitting your corrected revised manuscript and additional files. These have been uploaded successfully. We will continue processing the submission in the next few days, and will be in contact with an update once we have finished our remaining Editorial Checks.

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- English_editing_receipt.pdf
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1 ORIGINAL RESEARCH

2 Hendrik Setia Budi et al

3 **Estimation of Platelets Count and Bleeding Time of Mice**

4 **Treated with *Musa paradisiaca* var. *sapientum* (L.)**

5 ***Kuntze* Extract**

6 Hendrik Setia Budi¹

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8 Silvia Anitasari^{4,5}

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10

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26

27 **Abstract:**

28 **Objective:** the aim of this study was to estimate the platelets count and bleeding time on
29 peripheral blood smear of mice tail wound using *Musa paradisiaca var. sapientum (L.) Kuntze*
30 (ambonese banana stem extract).

31 **Design:** Randomized post-test only control group design.

32 **Materials and Methods:** Twenty-four male mice (*Mus Musculus*) were randomly divided into 4
33 groups. A negative control group was treated with carboxymethyl cellulose (CMC), a positive
34 control group (K+) treated aspirin 100 mg/kg body weight, group P1 treated with aspirin 100
35 mg/kg body weight and tranexamic acid 50 mg/kg body weight, and group P2 treated with 30% of
36 ambonese banana stem extract (ABSE). The mean and standard deviation data of platelets
37 counts and bleeding time were analyzed by one-way ANOVA statistical software.

38 **Results and Discussion:** Tranexamic acid had no significant effect on platelets count compared
39 to CMC group ($p = 0,871$), but administration of aspirin resulted in low platelets count significantly
40 ($p = 0.003$). The platelets count of ABSE and CMC groups were not significant different ($p =$
41 0.937). Aspirin has significantly shown prolonged bleeding time than CMC. tranexamic acid, and
42 ABSE groups. However, there was no difference between the tranexamic acid and ABSE group
43 ($p=0.934$). The bleeding time of tranexamic acid and ABSE group was similar, although the
44 platelets count in the ABSE group was lower than in the CMC group.

45 **Conclusion:** This study proved that ambonese banana stem extract has a potency to shorten the
46 bleeding time in mice tail wound without interfering to platelets count.

47 **Keywords:** haemorrhage, health risk, herbal medicine, peripheral blood smears, wound

48

49 **Introduction**

50 Wound is the loss or damage of some body tissues caused by a sharp or blunt trauma,
51 temperature changes, chemicals, burning, electric shock, or animal bites. This damage causes
52 significant morbidity rates worldwide, therefore its management is needed to effectively facilitate

53 the normal healing process for quick recovery.¹⁻³ Open wounds on the body surface cause rupture
54 of blood vessels leading to bleeding. In mild cases, the haemorrhage stops quickly, but when it is
55 wide and deep enough, it takes longer time to stop.⁴ A severe bleeding can cause impaired
56 perfusion, leading to tissue hypoxia which can cause hypovolemic shock.⁵ This condition is due to
57 insufficient blood in the circulation. Therefore, hypoperfusion (low oxygen perfusion) disrupts cell
58 metabolism, and reduces Adenosine Triphosphate (ATP) formation causing general organ
59 failure.⁶

60 Post-extraction teeth haemorrhage can cause severe complications such as uncontrolled
61 bleeding and infection. Wounds after tooth extraction should be well dried because there are
62 various kinds of infectious bacteria in the oral cavity.^{7,8} Blood clotting is a series of complex
63 process to achieve hemostatic state that prevents excessive blood loss after injury.⁹ When
64 bleeding occurs, there are three main processes of hemostasis and coagulation which includes
65 vasoconstriction, platelet aggregation, and activation of blood clotting factors. Therefore, platelets
66 have a major role in the hemostatic phase by forming blood plugs and activating the clotting
67 factors.^{10,11} Hence, the significance of hemostatic analysis to estimate platelet function, which
68 could be done on a peripheral blood smear under a light microscope.^{12,13}

69 However, severe haemorrhage can occur in patients who consume blood-thinning
70 aspirin.¹⁴ Hence, adequate bleeding control can be done by administering systemic hemostatic
71 drugs such as tranexamic acid.¹⁵ Several studies have shown that the administration of
72 hemostatic drug in postoperative patients can have side effects such as nausea, vomiting,
73 headaches, and allergic reactions.¹⁶ In fact, these medications are contraindicated in stroke
74 patients. Because the usage of existing coagulants is associated with evident side effects, new
75 coagulants should be created with a focus on medicinal plants that have relatively few adverse
76 effects.¹⁷

77 There are several plants with medicinal properties which can be used to heal wounds and
78 prevent infections with minimal side effects, therefore they are applied as therapeutic drugs.
79 Besides, it has been found that those plants with active ingredients can play important role in
80 wound healing. In Indonesia, banana trees have been proven contains saponins, flavonoids,

81 alkaloids, tannins and lectins which have various health benefits such as antihypertensive and
82 antidiabetic effects. Furthermore, ambonese banana sap has various therapeutic effects as an
83 antioxidant, antibiotic, and wound healing accelerator.¹⁸ The application of ambonese banana
84 stem sap at a concentration of 30% accelerated wound healing by increasing the expression of
85 Platelet-Derived Growth Factor (PDGF-BB).¹⁹ Effects of PDGF-BB topical treatment in animal
86 models have been shown to improve tissue repair under conditions of delayed wound healing.²⁰
87 Therefore, the aim of this study was to estimate the platelets count and bleeding time on
88 peripheral blood smear of mice tail wound using Ambonese banana stem extract (ABSE) as a
89 medicinal plant in hemostasis.

90 **Material and methods**

91 ***Samples and ethical clearance***

92 This study has passed the ethical clearance of health research no. 586 / HRECC.FODM / IX /
93 2019 Faculty of Dental Medicine, Universitas Airlangga. In compliance with Government
94 Regulation of the Republic of Indonesia Number 95 of 2012 Concerning Veterinary Public Health
95 and Animal Welfare. The procedures and methods that have been prepared based on the
96 consensus of all parties concerned with due regard to the requirements for safety, security,
97 health, environment, development of science and technology, and experience of both current and
98 future developments in order to obtain the maximum benefit possible. We used 24 male mice
99 (*Mus Musculus*), weighing between 20-30 g body weight (BW). Sample size formula was
100 obtained from health studies formulation as amount 6 samples each group. In the negative
101 control group (K-), the mice were given carboxymethyl cellulose (CMC) orally. Meanwhile, the
102 positive control group (K+) was given 100 mg/kg body weight aspirin dissolved with 1% CMC
103 orally for 7 days. The treatment group-1 (P1) was given aspirin dose of 100 mg/kg body weight
104 dissolved with 1% CMC orally for 7 days and given 50 mg/kgBW kg body weight of tranexamic
105 acid orally 30 minutes before cutting the tail. The treatment group-2 (P2) was given aspirin

Commented [hsb1]: We added this legend for positive control group

106 dissolved in 1% CMC orally for 7 days and given ABSE at concentration of 30% 30 minutes
107 before cutting the tail. The solution volume given orally was 1 mL/10 g body weight.

108 ***Preparation of ambonese banana stem extract (ABSE)***

109 The stems were obtained from the Plant Conservation Center in Purwodadi Botanical Gardens, at
110 Pasuruan. The plant was identified at LIPI (Indonesian Institute of Sciences) no. 1272 / IPH.06 /
111 HM / XI / 2019, which confirmed it as ambonese banana. The selected plant was 2.5-3 m high
112 with a diameter of around 17-18 cm. Furthermore, the age was between 12-13 months. The
113 identified stem sap was used according to the previous study.¹⁹

114 The stem sap was taken by cutting the bottom and then washing it to remove dirt.
115 Afterwards, they were cut into small pieces with a sterile knife, and distilled water of 200 ml was
116 added till they blend.²¹ A homogeneous banana stem sap and aquades were filtered using a
117 bugner funnel connected to a vacuum pump (Gast brand, USA) and given whatman filter paper
118 number 41. The resulted extract was stored in a closed dark bottle to reduce oxidation.²²

119 ***Platelets count analysis on peripheral blood smears***

120 Blood samples are taken through a scalpel cut near the tail tip. The last drop of blood was rubbed
121 on a glass object. The platelets count based on the results of peripheral blood smears was then
122 calculated using a counting chamber. The smear was stained with giemsa (Sigma-aldrich, USA)
123 and observed in five visual fields with a 400x magnification microscope (Nikon H600L, Japan).
124 The blood was placed at a distance of 2-3 mm from the end of the glass. Other smears were
125 made with a different glass and placed at an angle of 30-45 degrees in front of the blood droplets.
126 The smears were left to dry and labeled according to the treatment group. Giemsa dye was
127 diluted with buffer (1:4), then colors were mixed with methanol until it flooded the preparation. The
128 results were then observed in five visual fields under a microscope at 400x magnification.²³

129 ***Tail bleeding time analysis***

130 Bleeding time test was performed on experimental animals. The mice's tail was cleaned with 70%
131 alcohol and cut at a point of 2 cm from the tip using surgical scissors. The dripping blood was
132 absorbed on whatman absorbency paper. Meanwhile, the time from the first blood drip until it
133 stopped was calculated as the bleeding time. Furthermore, the blood was allowed to drip
134 spontaneously and placed on 16 absorbent paper boxes every 15 seconds.²⁴

135 ***Statistical analysis***

136 The data was analyzed using *Statistical Package for the Social Science Software* (SPSS). It was
137 presented in the form of *mean ± standard deviation* ($\bar{x} \pm SD$). In addition, the normality test of
138 platelets count and bleeding time data used *one sample kolmogorov smirnov*, while the
139 homogeneity used *Levene Test*. The mean difference between groups was analyzed using *one*
140 *way ANOVA* test and *least significant difference (LSD)* at 95% confidence interval.

141 **Results**

142 The data were presented in the form of figures, tables representing mean ± standard deviation (\bar{x}
143 ± SD), and graphs. Furthermore, statistical analysis was used to test the hypotheses.

144 ***Phytochemical analysis of ambonese banana stem extract***

145 We have provided the qualitative phytochemical analysis on the active substances in the stem
146 extract. Positive results were obtained from the flavonoid, tannin and saponin tests. The flavonoid
147 test was positive because the solution color changed to yellowish-orange. Moreover, the tannin
148 test was positive because there was a color change to dark-green after adding FeCl₃ reagent.
149 Furthermore, the saponin test was positive because there was visible foam formation that was
150 stable for about 10 minutes (Figure 1).

151 **Analysis of platelets on peripheral blood smears**

152 The mean of platelets count from 24 mice samples showed 163.3 in CMC group, 78.0 in aspirin
153 group, 169.7 in tranexamic acid group, and 163.0 in ABSE group. Based on these data, the
154 application of tranexamic acid and ABSE increased platelets around the wound compared to the
155 aspirin group, and equal to the CMC group (negative control) (Table 1).

156 According to Table 1, there was a significant difference between groups in platelet count
157 ($p = 0.000$). Tranexamic acid had no significant effect on platelets count compared to CMC group
158 ($p = 0,871$), but administration of aspirin resulted in low platelets count significantly ($p = 0.003$).
159 The platelets count of ABSE and CMC groups were not significant different ($p = 0.937$). The
160 peripheral blood smear with giemsa staining was examined and counted under a light microscopy
161 (400x) (Figure 2).

162 **Effect of ABSE on the tail bleeding time**

163 The bleeding time of CMC group was 115 secs, and aspirin group was 232.5 secs. It means that
164 bleeding time of aspirin group was longer than negative control group (CMC). Administration of
165 tranexamic acid and ABSE on mice tail wound shorten the bleeding time. In addition, the bleeding
166 time of ABSE group was 95 seconds, it means that it is shorter than that of aspirin group.
167 Therefore, the bleeding time of ABSE was similiar to the bleeding time of tranexamic acid group.
168 It means that ABSE has a great efficacy as a hemostatic agent (Table 2).

169 Based on *One Way ANOVA* test results, a significance value of $p = 0.005$ ($p < 0.01$) was
170 obtained. This means that there were significant differences in bleeding time. Furthermore, there
171 was a marked difference between the CMC and aspirin groups ($p = 0.004$) ($p < 0.01$). Meanwhile,
172 there were significant differences between the aspirin and tranexamic acid groups ($p = 0.004$) (p
173 < 0.01) beside the significant differences between the aspirin and ambonese banana stem extract
174 groups ($p = 0.006$) ($p < 0.01$). However, there was no significant difference between the
175 tranexamic acid and ambonese banana stem extract groups ($p=0.934$). Aspirin has significantly
176 shown prolonged bleeding time than CMC ($p=0.004$), tranexamic acid ($p=0.004$) and ABSE

Commented [KS2]: Table 1: Table 1 contains an indicator (a-d) but this is not clarified within the descriptive table footnotes. Please ensure all included indicators are described.

Please also supply a clean copy of table 1.

Commented [hsb3R2]: Thank you for sugesst, but we didn't mention "a-d" legends. We have explained in the footnotes which the different letter in superscript indicated significantly different. Example, the value "a" and "b" is significantly different, but "a" and "ac" is not significantly different.

Commented [KS4]: Table 2: Table 2 contains an indicator (a-d) but this is not clarified within the descriptive table footnotes. Please ensure all included indicators are described.

Please also supply a clean copy of table 2.

Commented [hsb5R4]: Thank you for sugesst, but we didn't mention "a-d" legends. We have explained in the footnotes which the different letter in superscript indicated significantly different. Example, the value "a" and "b" is significantly different, but "a" and "ac" is not significantly different.

177 (p=0.006), however, there was no difference between the tranexamic acid and ABSE group
178 (p=0.934).

179 **Discussion**

180 Ambonese banana stem sap contains tannins, flavonoids, and saponins as antibacterial
181 components.²² They also stimulate new cell growth in wounds. This sap can accelerate the re-
182 epithelialization process of epidermal tissue, formation of new blood vessels (neocapilerization)
183 and connective tissue (fibroblasts), and the infiltration of inflammatory cells in wounds. The sap
184 active substances that facilitate blood clotting are flavonoids, tannins and saponins.²⁶ Flavonoids
185 and saponins play a vital role in inhibiting the cyclooxygenase cycle by decreasing the production
186 of prostaglandins, which are responsible for vasodilation.²⁷ Tannins can also facilitate platelets
187 adherence to sub-endothelial blood vessels, hence help release adenosine diphosphate (ADP)
188 and thromboxane (TXA2) mediators.²⁸

189 The active compounds in ambonese banana stem sap have a role in the process of
190 hemostasis which is important to stop bleeding. There are many cells that play a role in this
191 process such as platelets, which are activated by collagen exposure. Therefore, the glycoprotein
192 on the cell surface adheres to the epithelium, which is mediated by von Willebrand Factor (vWF).
193 The adherence to the epithelial surface activates the membranes of alpha granules to release
194 TXA2 and ADP which are considered major factors in platelets aggregation process. Hence,
195 reducing the bleeding time of wounds or ruptured vessels. After aggregation, a coagulation
196 cascade is initiated which is influenced by extrinsic and intrinsic factors.¹¹ Ambonese banana
197 stem sap contains active compounds that are proven to accelerate wound healing. This is
198 achieved by increasing PDGF-BB which is a growth factor released during platelets aggregation
199 to activate the healing process. Therefore, when PDGF-BB production increases, it shows a high
200 level of aggregation.

201 This research showed longer bleeding time in the aspirin group compared to the CMC,
202 tranexamic acid and ambonese banana stem extract groups. The aspirin group was aimed to
203 prolong the bleeding time. It was reported that administering aspirin for 7 days prolonged

204 bleeding time.²⁹ This drug is a non-steroidal anti-inflammatory drug (NSAID) with blood-thinning
205 property through the inhibition of thromboxane A₂ (TXA₂).³⁰ Therefore, it inhibits blood
206 aggregation and causes prolonged bleeding time.³¹ The platelets count in the negative control
207 group was lower than CMC, tranexamic acid and ambonese banana stem extract groups.
208 Platelets count decreased in patients on aspirin medication, due to inhibition of aggregation,
209 leading to prolonged bleeding time.

210 The tranexamic acid group have shorter bleeding time, followed by those given the
211 Amboinese banana stem extract. These results are similiar to the previous study which stated
212 that tranexamic acid reduced bleeding time in patients who have been given aspirin.³² Meanwhile,
213 there was platelets increase in the tranexamic acid group. This occurred because the compound
214 is an anti-fibrinolytic drug that inhibits the breakdown of fibrin by plasmin through receptors found
215 on plasminogen. These receptors prevent plasmin from binding and finally stabilizes the fibrin
216 matrix.³³

217 Based on the results of the ambonese banana stem extract group, the bleeding time was
218 shorter than the positive control group with an increase in platelets count. Furthermore, a test
219 which stated that the use of 30% ambonese banana stem sap does not show any systemic
220 toxicity on visible tissues in the fibroblasts culture. However, it showed a large number of living
221 fibroblasts accelerated wound healing through the activation of PDGF-BB.¹⁹ The phytochemical
222 test results also showed that the stem sap contained active compounds, including flavonoids,
223 tannins and saponins, which have therapeutic effect in the hemostasis process.

224 The low platelets count in peripheral blood smears compared to the tranexamic acid
225 group is due to inadequate concentrations or dose, hence it affects the potency of the ambonese
226 banana stem sap in hemostasis. The negative control group that was given CMC showed a
227 normal hemostasis. Meanwhile, the positive control group that was given aspirin, tranexamic acid,
228 and amabonese banana stem extract had a longer hemostatic process. The administration of
229 tranexamic acid and ambonese banana stem extract led to a faster coagulation and increased
230 platelets in the wound area, as shown through peripheral blood smears. Based on the discussion
231 above, the use of ambonese banana stem extract in this study gave an effect similar to

232 tranexamic acid as a hemostatic drug. Therefore, it can shorten the bleeding time through
233 platelets activation.

234 **Conclusion**

235 The application of ambonese banana stem extract (*Musa Paradisiaca* Var. *Sapientum* (L) Kuntze)
236 shorten the bleeding time in mice tail wound without interfering to platelets count. On the basis of
237 the finding in this study, we have determined that it is essential to separate and purify the active
238 chemicals that are found in plants. By utilizing the NMR technique, being can analyze the
239 chemical structure in order to identify the active molecules that are involved in the process of
240 blood coagulation. Certain blood coagulation parameters can be collected straight from the
241 systemic circulation, which will result in a higher degree of certainty and a reduction in the amount
242 of harm inflicted to animal models.

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249 **Disclosure**

250 The authors have declared that there is no conflict of interest.

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334 **Figure legends:**

335

336 **Figure 1** Phytochemical analysis of ambonese banana stem extract content. Flavonoid (A),
337 Tannin (B) and (C) Saponin.

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341 **Figure 2** The platelet count in a peripheral blood smear with giemza staining. A) CMC, B) Aspirin,
342 C) Tranexamic acid, D) Ambonese banana stem extract. Black arrow: red blood cells; Red arrow:
343 platelets.

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