Author's response to reviews

Title: Evaluation of the wound healing property of Commiphora guidottii Chiov. ex. Guid.

Authors:

- Michael Gebrehiwot (mgebrehiwot@gmail.com)
- Kaleab Asres (kaleab.asres@aau.edu.et)
- Daniel Bisrat (daniel.bisrat@aau.edu.et)
- Avijit Mazumder (avijitmazum@yahoo.com)
- Peter Lindemann (peter.lindemann@pharmazie.uni-halle.de)
- Franz Bucar (franz.bucar@uni-graz.at)

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Author's response to reviews: see over
Response to Reviewer I: mohammadreza akhoondinasab

Reviewer's report:

i. I believe that methods of the article are precise and complete but it seems that bulk of it is more than usual and it is better to be deleted some unnecessary paragraphs.

Response: We thank the reviewer for his positive response. We believe that the manuscript is a bit too long not because we included unnecessary information but it is a comprehensive study which includes reports on composition of essential oil, fingerprint of the resin, wound healing effect, antibacterial and antifungal activity and anti-inflammatory actions. However, we have reduced the discussion mainly by excluding basic background information on some aspects of wound healing, antimicrobial and anti-inflammatory agents.

Response to Reviewer II: Mohamed Ahmed

Reviewer's report:

i. The study clearly clarified the content of essential oil and the resin of Commiphora guidottii Chiov. ex. Guid. The results are clear and well presented. However, it would be better if the authors conducted histological studies to show the changes in the tissue architectures at different stages of wound healing. In addition, it would be better to include some molecular studies, which could explain the mechanism of action to mediate the wound healing-enhancing effects of tested materials. For example, to study the expression of growth factors (Fibroblast growth factor, Vascular endothelial growth factor, Transforming growth factor beta, HGF,...) and inflammatory cytokines (Interleukin-1 beta, tumor necrosis factor alpha), particularly the authors referred to these cytokines in the discussion section.

Response: We appreciate the comments. Unfortunately we have not done the above. The reviewer might have realized that the manuscript is already too bulky due to the comprehensiveness of the study; this has also been commented upon by other reviewers. We have removed the part we discussed on growth factors and mentioned the above suggestions as the work that should be done in future work.

ii. Figure 3 does not contain any symbols indicating statistical significance as referred in the results section.

Response: Statistical significance is now shown using asterisk.
iii. In Table 3, where the control value (period of epithelization in non-treated wound).

Response: There is no non-treated group here. There is “left untreated group” in incision wound model only, and that was used to calculate the tensile strength of simple ointment group.

iv. The discussion section should be more concise.

Response: We have reduced the discussion part mainly by removing basic background information on some aspects of wound healing, antimicrobial and anti-inflammatory agents.

v. The manuscript should be revised for grammatical and spelling errors, for example: Page 19, line 16 (significant reduction should be a significant reduction), page 20, line 2 (Antiinflammatory should be Anti-inflammatory), Page 23, line 3 (indicate should be indicates).

Response: We have addressed the corrections suggested and other minor errors committed.

Response to Reviewer III: Fabio Santos

Reviewer’s report:

i. Major compulsory revisions. On the other hand, the use of anesthetic method needs a strong justification. Ether like inhalatory anesthesia is not acceptable methods for international committees.

Response:

i. Diethyl ether was used because it is long acting and slow onset of action. Having slow onset of action is advantageous because it greatly increases the margin of safety during the experiment. It is also reliable and potent anaesthetic.

ii. There was enough personal protective equipment during the experiment; like face mask, to avoid the toxicity of diethyl ether.

iii. Appropriate precautions were made to avoid explosion and flame. One of the precautions was using a small volume of ether.

iv. Unlike many other anaesthetics which can lead to immunosuppression, diethyl ether has a smaller impact on the receptors located in the membrane of erythrocytes maintaining a stable immune function in mice. This is a big advantage to our work since immunosuppression could have affected our results.
v. Although diethyl ether can cause irritation in mucosal membranes, it has an advantage including quick return from anaesthetic hypothermia, lower incidence of death and relative technical ease of administration.

Response to the General comments

Methods:
Plant Material:
Page 6, line 1: I would like to see more details about plant material: Where and when this plant material was obtained? Place and month of the year are important for plant material characterization.
Response:

Details about the plant material have been incorporated in the main text.

Experimental animals:
What is the sample size for Mice and Rats? Males and females? What is the animals’ age?
Queries have been addressed in the revised version.

Skin irritation test:
Page 9, Line 12. Two groups containing 3 animals are enough for the statistical analysis? Justify.
Please, indicate the experimental groups clearly.
Was there any kind of examiner calibration for the Draize dermal irritation scores? I think is very difficult to obtain a real concordance for erythema scores 2 – 3 and for edema scores 2 – 3 and 3 – 4. The authors need to include the examiner calibration process and intra/inter-examiner concordance (kappa test). Otherwise, skin irritation results could be not reproducible
Response:
It was typographical error. The number of animals used was 6 per group. That has been corrected in the main text.
Two areas on the back of each rat on each side of the vertebrae (1 cm from the midline of the vertebral column) were shaved and were marked before the experiment. One area was for test sample ointment and the other was left untreated to be used as a comparison. Therefore, there were only two groups of animals: one for the resin and the other for the oil. There was an area left untreated with the ointments of the oil and the resin on each animal. We have now stated this clearly.
There was only observer and that could be one drawback of the study.

In vivo wound healing models:
Excision wound model: Page 11, Line 8. Please, indicate the four experimental groups clearly.

Response:
Part of the paragraph has been rephrased to clarify the procedure followed.

Page 11, Line 8. “…anesthetized with diethyl ether…” Nowadays, use of ether as an anesthesia agent in animals is not allowed without strong scientific justification. Main reasons: Ether is flammable and forms explosive mixtures with oxygen and room air. Anesthetic induction may cause distress in animals due to ether’s irritant properties, which have been shown to cause coughing, profuse bronchial and salivary secretions and laryngospasm. Ether can also cause pre-existing subclinical respiratory disease to develop into acute severe infection following recovery from anesthesia. Induction and recovery times for ether anesthesia are relatively slow.

Response:

i. Diethyl ether was used because it is long acting and slow onset of action. Having slow onset of action is advantageous because it greatly increases the margin of safety during the experiment. It is also reliable and potent anaesthetic.

ii. There was enough personal protective equipment during the experiment; like face mask, to avoid the toxicity of diethyl ether.

iii. Appropriate precautions were made to avoid explosion and flame. One of the precautions was using a small volume of ether.

iv. Unlike many other anaesthetics which can lead to immunosuppression, diethyl ether has a smaller impact on the receptors located in the membrane of erythrocytes maintaining a stable immune function in mice. This is a big advantage to our work since immunosuppression could have affected our results.

v. Although diethyl ether can cause irritation in mucosal membranes, it has an advantage including quick return from anaesthetic hypothermia, lower incidence of death and relative technical ease of administration. Also, the described effects on bronchial system are not directly related to the wound healing properties measured.

Page 11, Line 9. Why 540 mm²? How the wound size was defined? How the wound area was standardized?
Response:
The wound was created with a circular disc of area 540 mm² by which the area of the disc was determined by using graph paper. The area was just selected arbitrarily after reviewing other similar research works.

Was the wound made in the rat dorsum? Please address this information clearly.
Response:
The wound was made on the rat dorsum.

The animals of each group were kept together? The animals could lick the dorsum from one another and remove the products that were being tested.
Response:
The animals were kept in an individualized cage.

Page 11, Line 11. “…basis till the wound healed completely…” How long the completely wound healing occurs in the rat?
Response:
When we say the wound healed completely, we mean that there is complete falling of scab. However, the complete wound healing time varies due to individual variation, whether the animal was treated or not, and with what the animal was treated.

Incision wound model:
The authors need to include more details about experimental procedure
Response:
Details have been included in the revised version.

Results:
Toxicity study:
Page 17, Line 7: “…resin killed more than 50% of the animals…” But the sample size is only 3 animals? Explain.
Response:
It was typographical error. Actually 6 animals were used.

Wound contraction and period of epithelization:
Page 17, Line 22: What does it mean “negative control”? No treatment or placebo?
Response:
Negative control means the placebo group. There is “left untreated group” only in the incision wound model.

Table 3: Was there statistical difference between Essential oil “versus” Resin; Essential oil “versus” Nitrofurazone; and Resin “versus” Nitrofurazone?
Response: There was no significant difference between nitrofurazone and resin, between nitrofurazone and the oil, and between resin and the oil. This is clearly stated in the revised version.

Tables 7 and 8: Please include SD for each mean value.

Response: MIC was expressed as an average from three independent experiments, each performed in triplicate.

Q. Discussion:
Page 23, Line 8: Misunderstanding concept: negative control different of placebo control. Please check it.

Response: the negative control is the placebo group

Q. What are the clinical implications?
Both the strengths and weaknesses of the observations should be discussed.

Response: we observed the effect of the formulated ointment of C. guidotti oil and resin on the different aspects of wound healing, both in vivo and in vitro, that are of great clinical significance. Given the positive effect on these aspects of wound healing process (i.e. anti-inflammatory, antimicrobial, wound contraction, period of epithelization and tensile strength), our observation provided a promising data which support the use of the formulated ointment as an alternative agent for wound management. Yet some of the observation (skin irritation test and period of epithelization determination) were subjective; and this, to some extent, may affect the reproducibility of the experiment. This is indicated in the revised version.

Figures:
Page 39. Calibration curve, figure 1 is unnecessary.

Response: Figure 1 is removed.

Figure 3: Line in each bar is SD or SEM? Include statistical results in this figure

Response: Figure 3: Line in each bar is SEM.