Author's response to reviews

Title: Tannin extracts from immature fruits of Terminalia chebula Fructus Retz promote cutaneous wound healing in rats

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Author's response to reviews: see over
Reviewer: Shivananda Nayak
Revised sentences were highlighted in red color in manuscript.

1 The title must be revised as suggested below:
   Tannin extracts from Immature fruits of *Terminalia chebula Fructus Retz.* promote cutaneous wound healing in rats.

2 **Abstract**
The abstract is poorly written and complete abstract to be rewritten

**Background:** Tannins extracted from immature fruits of *Terminalia chebula Fructus Retz.* are considered to be effective components to promote wound healing. This study aims to study optimal extraction and purification technology (OEPT) of tannins and study the use of the drug for the treatment of a cutaneous wound of rat as well as its antibacterial effects.

**Methods:** The content of tannin extracts was measured by the casein method and antibacterial ability was studied by the microdilution method in vitro. In wound healing experiment, animals in group Ⅰ, Ⅱ and Ⅲ were treated with vaseline ointment, tannin extracts(tannin content:81%) and erythromycin ointment 5mg per wound, respectively. Selected pharmacological and biochemical parameters were used to evaluated wound healing.

**Results:** Content of tannin extracts after optimal extraction and purification was increased to 81%. Tannin extracts showed inhibition of *Staphylococcus aureus* and *Klebsiella Pneumonia* in vitro. On day 7 and 10 after excision wounds creation, the percent of wound contraction of group Ⅱ was higher than that of group Ⅰ. On day 3, 7, and 10 after wounds creation, the wound healing quality of group Ⅱ was better than that of group Ⅰ in terms of granulation formation and collagen organization. On day 3 after wound creation, the vascular endothelial growth factor expression of group Ⅱ was higher than that of group Ⅰ.

**Conclusion:** The results suggest that tannin extracts from dried immature fruits of can promote cutaneous wound healing in rats, which probably results from a powerful antibacterial and angiogenic activity of the extracts.

3 **Introduction**
Authors should clearly state the reason for selecting tannins from *Terminalia chebula Fructus Retz* to show the wound healing activity

   It has been accredited for a long history of use in Ayurvedic and also Thai folk medicines as a natural remedy for skin diseases, wound and rejuvenation[7]


4 **Why authors selected only two microorganisms to study the antimicrobial activity?**
   The research group has been dedicated to the research on natural drugs for the treatment of infected wound, the most typical clinical wound infection is caused by *Staphylococcus aureus*, however, Gram-negative *Klebsiella* has emerged frequently in China’s clinical infected wound reports over recent years, and the *Klebsiella* is drug-resistant and is also the strain detected mainly in superbacteria. Furthermore, the research group is quite familiar with the culture of the two bacteria and the action mechanism of antibacterial drugs on them, so these two bacteria are selected.

5 **Discussion**
Include some hypothetical explanation for the wound healing activity of tannins
from Terminalia chebula Fructus. For this refer some recently published articles by Nayak BS et al
insert two references into discussion.

**6 Why authors suddenly jumped to say murine instead of rats, justify**
We have corrected this mistake as followed:
Tannin extracts from *Terminalia chebula Fructus Retz.* can promote cutaneous wound healing in rats, which probably results from a powerful angiogenic and antibacterial activity.

**7 References Not uniform and format as per the journal rules**
We have corrected format as rules

**8 Table 4:**
**On day 14 vaseline treated group also showed 96% wound contraction when compared to test and standard group which showed 100%.** 96 and 100 are almost nearer. Justify this

On day 14 after excision wounds creation, the percent of wound contraction of group I was almost equal to that of group II because of self-healing in rats. So there is no significant difference between three groups.
Reviewer2
Revised sentences were highlighted in pink color in manuscript.

① Comments:
1. What is the concentration of tannin used locally in the study? This observation should come in the abstract.
2. What do you mean by study group? Write clearly

   1. In wound healing experiment, animals in group I, II and III were treated with vaseline ointment, tannin extracts (tannin content: 81%) and erythromycin ointment 5mg per wound, respectively.
   2. Study group refers to group II which was treated with tannin extracts.

② Comments: Authors have used only single model of excision wound in rats so they should use term models. Yellow coloration indicates correction while words painted in red need removal.
I have corrected the manuscript according to the reviewer’s opinion.

③ Comments: Quantity of water and ethanol is not mentioned. Please give detailed methodology.
The immature fruit powder of Terminalia chebula Fructus Retz. (10g in weight) was extracted with water (100ml) at 50°C.
The extracts were added ethanol of 95% concentration and the concentration of extract solutions were diluted to 80%. The extract solution was deposited for 12 hours and centrifuged at 4000rpm for 10 min.

④ Comments: .....were diluted to a volume of 12 ml with 29% Na₂CO₃ solution? and were diluted to a volume of 25ml with 29% Na₂CO₃ solution.

⑤ Comments:
1. In most of the illustrations group I is always a negative control group while II, III etc. are treated groups. So the sequence of data should be changed and introduced in the text.
2. The dose of test drug, tannin and reference drug, erythromycin and Vaseline base is mentioned 5mg. It seems that authors have dispersed their test drugs in Vaseline base so they should use the term % concentration in vaseline base which would come to 0.5% (5mgof tannin or erythromycin /g vaseline base (weight/weight)

   1. The sequence of data has been changed.
   2. The animals of group II were topically treated with tannin extracts at a dose (25 mg/kg/day), so a rat was treated with tannin extracts 5mg per wound. The animals of group I and III were treated with Vaseline and erythromycin 5mg per wound.

⑥ Comments: the result has to be written again in light of various mistakes done
while editing the paper in light of the sequence of treatments.
The sequence of data has been changed.

7) **Comments: S. aureus is Gram +ve while K. pneumonia is Gram-ve**
I have corrected the manuscript according to the reviewer’s opinion.

8) **In order to study effective concentration of tannin extracts, we extracted and purified tannin. We used water and alcohol as dissolvents because requirement of industrialized production as well as safety, further determined the optimum extraction and purification technology (OEPT) of tannin extracts and ensured its quality (Sentence not clear).**

When we extracted and purified tannin, we used water and alcohol as dissolvents because requirement of industrialized production as well as safety, further determined the optimum extraction and purification technology (OEPT) of tannin extracts in order to ensure its high content of tannin.