Author's response to reviews

Title: Antibacterial effect of crude extract and metabolites of Phytolacca americana on pathogens responsible for periodontal inflammatory diseases and dental caries

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Author's response to reviews: see over
Aug. 20, 2014

Dear Editor-in-Chief,

Sir, I am herewith submitting the revised manuscript entitled “Antibacterial effect of crude extract and metabolites of Phytolacca americana on pathogens responsible for periodontal inflammatory diseases and dental caries” for consideration for publication in the esteemed journal “BMC Oral Health”.

The manuscript was revised accordingly to the reviewers’ comments. We scrutinized the comments, and tried to answer as much as we could. Please find the attached file for the responses to the reviewers comments, which provided all answers for the comments raised by the reviewers. Our manuscript was much improved by the revision asked by the reviewers’ critical comments.

If you have more comments regarding to this revised manuscript, please feel free to contact me through phone (82-53-810-3029) or E-mail (khbaek@ynu.ac.kr). We welcome all of your comments, which definitely help to improve the quality of our manuscript.

Sincerely Yours,

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Author’s response to Reviewers’ comments

We appreciate the editor’s kind comments. We prepared our answers for the comments as follows;

1. The authors should clearly mention the solvent finally used for dissolving the crude extracts and other extracts /compounds during assays for antibacterial activity were done.

Five % of dimethyl sulfoxide (DMSO) was used for dissolving the crude as well as other fractions for antibacterial assay. We added the information in our manuscript (Line 122).

2. In the antibacterial activity assay the authors have used only medium control but they must also include solvent controls for each extract/compound.

We added the information about the solvent control in our manuscript (Line 137).

3. Since it is a research article, structures of the compounds does not merit to be in main figure. Rather, the authors should include graph for OD values of antibacterial activity of control as well as experimental samples in the main figure.

As per the reviewer’s kind comment, we deleted the Fig 1 for the structures of compounds from the manuscript. Therefore, Figure 2 is automatically changed to Figure 1. The Figure 1 represents the percentage activity of different compounds against the pathogens that was calculated from the O.D values.

4. In the discussion section the authors have discussed the mechanism of action of the biologically active extracts against one gram negative bacteria *P. gingivalis*. They should also discuss why the same mechanism will not be applicable for another gram negative bacteria used in the study? *E. coli* DH#
We appreciate the editor’s comment. We discussed the different mode of actions between \textit{P. gingivalis} and \textit{E.coli} strain in the discussion part (Line 222-224).

5. \textbf{In the Figure-2 the authors should include the actual name of the compounds in each graph (Diagonally below the x-axis), instead of using p1, p2\ldots to make it more legible.}

   We modified Figure 2 (after deleting the Fig. 1, the Fig 2 became Fig. 1 in the revised manuscript) as per the reviewer comment.

6. \textbf{In the Figure-2 A,B, C are 3 different graphs hence the concentrations should be inserted in each graph}

   We modified Figure 2 (after deleting the Fig. 1, the Fig 2 became Fig. 1 in the revised manuscript) as per the reviewer comment.

7. \textbf{The authors should include activity against non-pathogenic gram positive bacteria.}

   We appreciate the critical question raised by the esteemed editor and reviewer. In most of studies for the oral pathogenic microorganisms, the antimicrobial activity against \textit{E. coli} was tested because \textit{E. coli} is commonly found in many parts of human body and traditionally used as the control representing non-pathogenic bacteria. Thus we just checked the antimicrobial activity against only the gram negative \textit{E. coli}. We did not plan to do the experiments testing non-pathogenic gram positive bacteria because traditionally only the oral pathogenic bacteria and a non-pathogenic bacterium \textit{E. coli} have been tested for finding the controlling effect of natural compounds on the oral pathogens.

   Though we did not include the experiments testing the crude extract on a non-pathogenic gram positive bacterium, however, the main purpose of this manuscript was revealing the controlling effect of the \textit{Phytolacca} extract on the oral pathogens, not on non-pathogenic bacteria. Therefore, please accept our excuse of not including the activity against non-pathogenic gram positive bacteria in this manuscript.
8. Methods: Authors have mentioned the identification of *P. americana* L. by taxonomist and storing of the specimen. It will be better if authors provide the name of the identifier and voucher number or the passport data of specimen deposited.

   We included the taxonomist name and the voucher number in the revised manuscript (Line 113, Line 115).

9. Methods: Authors should specify which part of the plants they consider as aerial part, that are used for the preparation of plant extracts. Also, Authors have not cited any reference for preparation of crude plant extract. Is this methods of extraction has been validated or standardized earlier by authors

   We provided the information of the aerial part composed of stems and leaves (Line 111). We also added the reference for the extraction method in the revised manuscript (Line 121).

10. Results: The figure 2 can be compiled to one. The graphs shows no anti-bacterial activity of natural compounds present in *P. americana* below 4 µg/ml. Hence, representing them in the figure can be avoided. It will be better if authors can reframe the figure 2. Also, authors can use P4 and P5 instead of P5 and P6.

   We modified the Figure as per the comments in our revised manuscript.

11. Although the results indicates a significant anti-bacterial role of crude extracts of *P. americana*. Authors can further confirm the findings with agar diffusion assay and/or MIC, MBC assay.

   We included the MIC values for the extracts in the Table 1.

12. Line 118- 17.24% is the yield of the extract

   We changed the sentence in the revised manuscript (Line 118-119).
13. Line 95 and line 174 - seems to be contradictory as authors mentioning little evidence of the nature of bioactive compounds present in *P. americana* vs presence of different types of natural compounds.

We changed Line 93-96 in the revised manuscript as “The use of this plant in herbal medicine is typically based on its folklore and traditional evidence. Scientific evidence on the medicinal potential and some types and nature of bioactive compounds present in this plant has been reported [12].”. Therefore, there is no confliction between line 95 and line 174.

14. Line 189 - *E. coli*

We changed *E. coli* in the revised manuscript (Line 192).