Author’s response to reviews

Title: Anti-viral activity of culinary and medicinal mushroom extracts against dengue virus serotype 2: an in-vitro study

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Author’s response to reviews:

August 3, 2019
Dr. Esther Fagelson,
Editor,
Journal of BMC Complementary and Alternative Medicine

Dear Dr. Esther Fagelson,

Thank you very much for reviewing our manuscript. We greatly appreciate the editor and reviewers for their comments and suggestions. We had carefully reviewed the comments and had revised the manuscript accordingly. We had enclosed our point-by-point response to editor’s and reviewer’s concerns as below.

The followings are our point-by-point responses to editor comments:

1. Please read and address the final reviewer comments which can be found below. Please ensure that it is clear that your conclusions are from in vitro analysis and that more evidence is needed to prove the antiviral effect of the mushrooms.
Response: As suggested by editor, we had clearly stated in conclusion that this is an in-vitro findings and further studies need to be carried out to prove the anti-dengue effect in mushrooms. Please refer to page 22 & 23, line 530 to 537.

In conclusion, this is first study to reveal that mushroom aqueous extracts have an in-vitro anti-dengue effect via interfering the initial stage of DENV-2 infection which included the attachment and penetration of the virus through the cell membrane. Further research is needed to elucidate the structural characteristics of glucan and protein complexes that might trigger the anti-dengue effect, to prove the anti-viral effect of the mushroom on human monocytes and suitable murine model and to evaluate oral bioavailability and metabolism of selected doses by in vivo pharmacokinetics profiling that necessary for selecting potential anti-dengue candidate for future clinical development.

The followings are our point-by-point responses to reviewer Ying-Ray Lee comments:

1. The anti-DENV2 activity of the mushroom extract needs to confirm in human monocyte.
   Response: As suggested by reviewer, we had included a statement in conclusion section that anti-viral effect will be further tested in human monocytes, please refer to page 22 & 23, line 532 to 537. Further research is needed to elucidate the structural characteristics of glucan and protein complexes that might trigger the anti-dengue effect, to prove the anti-viral effect of the mushroom on human monocytes and suitable murine model and to evaluate oral bioavailability and metabolism of selected doses by in vivo pharmacokinetics profiling that necessary for selecting potential anti-dengue candidate for future clinical development.

2. The inflammatory cytokines and chemokines have to evaluate under mushroom extract treatment.
   Response: We agree with the suggestion by reviewer, we had conducted an in-vitro study on the anti-inflammatory effect of mushroom extract against inflammatory cytokine expressed in dengue infected monocytes, the findings of this study had been accepted for publication in Journal of tropical biomedicine under titled “Anti-Inflammatory effect of mushrooms in dengue-infected human monocytes”.

3. Mushrooms are edible as well as used by many populations as medicine. But is the dose used in this study biologically relevant? What is the rationale for choosing this dose? Because the authors use the extracts to prove antiviral activity, its applicability and relevance would be significant if the availability of the dose/active compound is biologically feasible considering oral bioavailability and metabolism.
   Response: As suggested by reviewer, we had mentioned the selection of the dose for antiviral screening in page 8, line 194-198.
   The maximum non-cytotoxic concentration (MNCC) was established from the concentration that showed least cytotoxic effect towards Vero cell compared with negative controls. The concentrations below MNCC were selected to evaluate of anti-dengue activity of mushroom extract using plaque reduction assay.

   The oral bioavailability and metabolism of this dose will be studied in in vivo pharmacokinetics study in near future, this statement had been included in conclusion section, please refer to page 22 & 23, line 532 to 537. Further research is needed to elucidate the structural characteristics of glucan and protein complexes that might trigger the anti-dengue effect, to prove the anti-viral effect of the mushroom on human monocytes and suitable murine model and to evaluate oral bioavailability and metabolism of selected doses by in vivo pharmacokinetics profiling that necessary for selecting potential anti-dengue candidate
for future clinical development.

We hope that you find our responses satisfactory and that the manuscript is now acceptable for publication.

Sincerely,

Ms. E. Kavithambigai,
Corresponding author