Author’s response to reviews

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

Authors:
Kavithambigai Ellan (e_kavi8@yahoo.com)
Ravindran Thayan (rthayan@gmail.com)
Jegadeesh Raman (ramanjegadeesh@gmail.com)
Kazuya Hidari (hidari@jc.u-aizu.ac.jp)
Norizah Ismail (norizahbtismail@yahoo.com)
Vikineswary Sabaratnam (viki@um.edu.my)

Version: 4 Date: 16 Jul 2019

Author’s response to reviews:

July 16, 2019
Dr. Deepa Nath,
Editor,
Journal of BMC Complementary and Alternative Medicine

Dear Dr. Deepa Nath,

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 Wildriss Viranaicken (Reviewer 3) comments:

1) The points were discussed and the manuscript was modified accordingly. I understand the difficulty of repeating the experiments, but please clearly indicate the number of repetitions for each of the experiments in the figure legends.
however, I don't understand the value table for the results of Q-PCR, it's not very clear. what do the columns and rows correspond to?
Response: As suggested by reviewer, the repetition for each experiment was mentioned in the legends for table and figure [Table 3 (pg. 14, line 327-328), Table 4 (pg. 15, line 351-352), Table 5 (pg 18, line 419), Figure 2 (pg. 13, line 730-731), Figure 3a & 3b (pg. 31, line 743-744)].

We had amended the table for RT-qPCR result, please refer to table for figure 3a and 3b.

The followings are our point-by-point responses to Ying-Ray Lee (Reviewer 4) comments:

1. Author claim that "Anti-Inflammatory effect of mushrooms in dengue-infected human monocytes" had been submitted for publication in Journal of tropical biomedicine in their response letter. In this study, mushroom extracts block the attachment and entry of DENV2 in Vero cells, and may through glucan and protein complex competition. Moreover, human monocyte has been well known to be the major infected cells in human. Therefore, authors have to confirm that mushroom extracts can reduce DENV-2 infection in human monocytes.
Response: We agreed with reviewer, vero cell was utilized for first line screening for anti-dengue activity of mushroom extracts. Extension of this project will be carried out which will include the study of human monocytes to evaluate anti-dengue of mushroom extract.

2. As the statement in the result "No significant correlation was found between the concentration of glucan or protein and anti-dengue activity, because mushroom specimens, even with low glucan or protein content, also exhibited prominent anti-dengue activity.", authors also suggested that "The anti-viral components might be glucan and protein complexes that are abundantly present in the extracts." in the abstract. It is recommended to revise this statement or authors have to explain why.
As the anti-tumor studies of a protein bound glycan isolated from mushroom of Polystictus Versicolor, PSK or Krestin (authors responses in the letter), these suggested that the glycoproteins on the cells may express in many types of cells and may play some important roles on the cell survival, and cellular physiology. Therefore, whether mushroom extracts are safe in the human and can be used as an effected therapeutic agent needs more studies.
Response: As suggested by reviewer, we had amended the sentence in the abstract "The anti-viral components might be glucan and protein complexes that are abundantly present in the extracts." Please refer to page 3, line 66-68.
The structural characteristics of glucan and protein such as molecular mass, branching degree, charge density, and molecular composition might play significant role in promoting anti-dengue effect in mushroom extract.

In page 22, line 521-526, we had included further explanation regarding the role of structural characteristics of mushroom polysaccharide and their anti-viral effect.
Ghosh et al., 2009 [44] had reported that the antitherpetic properties of sulfated polysaccharides are determined by a combination of structural features such as molecular mass, branching degree, charge density, and molecular composition of uncharged portions. Cardozo et al., 2011 [32] also had recommended that larger molecular mass and complexity of branching of sulphated polysaccharide of Agaricus brasiliensis are important for the inhibition of the herpes virus penetration.

As suggested by reviewer, we also had included few statements supporting the role of anti-cancer drug derived from mushroom proteoglycan and its potential as anti-viral compound. Please refer to page 20, line 481-485.
Mushroom protein bound glycan isolated from Polystictus Versicolor, PSK or Krestin which currently been used as adjunctive therapy for various types of cancers in Japan, showed promising potential as an anti-HIV agent, by downregulating viral replication and promoting the upregulation of specific antiviral chemokines (RANTES, MIP-1α/β, and SDF-1α) known to block HIV-1 coreceptors in THP1 cells and human PBMCs

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

Sincerely,

Ms. E. Kavithambigai,
Corresponding author