Reviewer's report

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

Version: 2 Date: 23 Dec 2018

Reviewer: Wildriss Viranaïcken

Reviewer's report:

This revised version of the manuscript describes the antiviral activity of fungus extract on DV2. The authors responded to the first remark of the editor and reviewers.

However, several points remain to be clarified:

1/ Cytokines are markers of inflammation and do not demonstrate an antiviral effect. The anti-inflammatory effect can have a beneficial effect when organisms are infected. For example, dexamethasone is an anti-inflammatory, and yet it is not an anti-dengue. Please modify accordingly. Also these results are presented in the discussion, they should be presented in the result section under a table and experimental procedure should be adapted (ELISA and macrophage infection).

2/ Figure 1 is incorrectly mounted. A graph with PFU would be more informative with statistics.

In general, the presentation of the results in table (CC50 and IC50) is synthetic, but seems to limit the ability of readers to clearly identify the effects described. Graphs presenting the data in dose response and with statistical analyses would be more informative and easier to interpret.

3/ In Figure 2, the experiment was done in n=2, so the shape of the graph is not adequate. No error bar and no significance on a repetition. Please change. n=3 will be fine.

This experiment is very important and suggests by comparing with the Plaque reducing assay, an action of the extract tested in figure 1 on the egress of the DV2. please complete and see the point 4 below.

4/ An MTT test alone seems to me insufficient to conclude on the cytotoxic effect moreover to make plaque reducing assay, the cells are incubated 7 days in the CMC with the extracts and the CC50 was tested over 48 hours. It is therefore necessary to make a cytotoxicity test with LDH assay, neutral red assay or ideally clonogenicity assay with violet crystal on a time course of more than 48 hours. Otherwise, it is necessary to review the flow chart of the plaque reducing assay experiment: Infect Vero cells for 1 hour, then treat the cells with the extracts for 48 hours, then determine the viral progeny in the culture supernatants by PFU and/or RT-qPCR. Do the RT-qPCR in parallel on the cells.

5/ Also the authors focus on glycans, proteins or polyphenols but aqueous extracts has many molecules of other nature capable of antiviral activity. A "time of addition" experiment must be carried out to determine whether the action is on the entry,
replication or egress. This experiment is crucial and important. These results will help to better value the chemical characterization in the discussion part.

6/ The part on the correlation between composition and anti-DV2 activity and must be straightforward because it reaches a dead end.
It is possible to test the major compound beta-D glycan in your tests or alternatively support your chemical composition analysis with recent study by Song et al, 2018 "Assessment of activity and mechanism of action of β-Dglucan against dengue virus" in Tropical Journal of Pharmaceutical Research.

7/ Line 562 to 570, just because the level of NS5 and E decreases does not mean that the extract can have an effect on replication or entry. Please change and see point 5.

8/ The manuscript must be reviewed for multiple type error.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

Yes

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I recommend additional statistical review

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