

RESPONSE LETTER

Editor

No.	Comments	Reply action taken	Line number
1.	<p>After careful consideration, we feel that your manuscript has merit but does not fully our Journal's publication criteria as it currently stands. Therefore, we invite you to submit a revised version of the manuscript that addresses the points raised during the review process, as written below this email and as the file attachments.</p>	<p>Thank you for your consideration to our manuscript. We have made improvements based on reviewers comments to fully meet the Journal's criteria. We have addressed points that have been raised by the reviewers.</p>	<p>Throughout the manuscript</p>

Reviewer A

No.	Comments	Reply action taken	Line number
1.	<p>In general, like every scientific manuscript written in English, for us non-English-speakers, the effort in making the manuscript to be submitted in a local or international journal is the same. Therefore, there is no excuse to give less effort in writing for local journals. It applies to everyone, including myself. we must encourage ourselves to give the same effort for every manuscript we write, regardless of the journal we submit in.</p>	<p>We tried our best to improve the manuscript in order to fit the journal's criteria. We have re-checked for typos, grammars, and ambiguities.</p>	<p>Throughout the manuscript</p>
2.	<p>The comment on the Abstract section is already reflected in the following sections after that.</p>	<p>We have revised the Abstract to reflect the changes advised.</p>	<p>Abstract section</p>
3.	<p>Introduction: I would like to ask the authors to add something</p>	<p>We have re-written the paragraph to include connector between sentences as advised by the reviewer, as follows:</p>	<p>Line 40 – 43</p>

	to connect these paragraphs. For example: “we need to think about the possibilities of generating antiviral compounds. One of them could come from the nature. Nature has been _____ etc” until we can connect it to the next paragraph.	“With the current inavailability of antiviral therapy for DENV infection, a search for compounds having antiviral effect needs to be established. One of the possible sources of compound with anti-DENV activities will be from the natural sources.”	
4.	Introduction: The authors do not need to add abbreviation if the terms only appear once in the manuscript.	We have removed the abbreviations.	Line 30 – 31
5.	Materials and Methods: It would be great if the authors could also provide the ATCC number	We have provided the ATCC number for the cells.	Line 82 – 84
6.	Materials and Methods: As far as I know, the authors do not need to put scientific name of the monkey in the manuscript. Just “The African green monkey Vero cells___” is enough in my opinion.	We tend to keep the scientific name to be consistent with the name of the other cell lines.	Line 84
7.	Materials and Methods: It is a must, I think, that the authors need to consistently write the same term. In this case, since the title is “6- gingerol”, then I think it is much better if everything is written as 6-gingerol, unless it is stated otherwise.	Thank you. For consistency we have revised all compound name to 6-Gingerol.	Throughout the manuscript.
8.	Materials and Methods: Why didn’t the authors use the same cell line for viability and antiviral activity experiments? Is there anything particular about this cell line?	The BHK-21 cell line was used in order to measure the titer of the DENVs in the antiviral assay system. This cell line has been routinely used in the plaque assay system since it gives good plaque morphology compared to other cell lines, making the titer calculation more feasible. We have added a reference for the use of BHK-21 cell in plaque assay.	Line 115

9.	<p>Results: I think the authors need to show the figure for this result. It is quite difficult to imagine such things without the aid of a good figure. The figure needs to show doses used in this experiment to avoid any confusion and the marker of significance, if any.</p>	<p>Agreed. We have added a Figure to show the effect of different concentrations of Curcumin and 6-Gingerol to A549 cells. A marker of significance (ANOVA statistic <i>p</i> values) has been added in the Figure 1 to better describe the data written in the Results section.</p>	Figure 1
10.	<p>Results: For figure 1 and 2, is it possible to add the marker of significance? For example (fig 1), in DENV-1, curcumin at 50 μM significantly reduced viral titer compared to control (medium only). Please also add the information of the cell line used in each particular experiment.</p>	<p>We have added statistical values measured using ANOVA statistics of each compounds groups for each DENV-serotype to describe the significant reduction of virus titer after compound treatment. Figures (now Figures 2 and 3) have been revised accordingly and information of the cell line used has been added in Figure legend.</p>	Figures 2 & 3 Line 142 - 170
11.	<p>Results: Does IC50 value belong to the virus? Or the compound? Overall, I understand the message in the table 1, however, I am wondering, why did the authors decided to make the mean of IC50 from four serotypes? Even though they are the same DENVs, but they are not entirely the same. I suggest that the authors show the results of IC50 per compound per serotype.</p>	<p>The IC50 values belong to the compound. We have modified Table 1 title to clarify the meaning. The mean values have been deleted and we agree with the Reviewer to only show the IC50 result per compound per serotype.</p>	Table 1
12.	<p>Results: Since there are four serotypes of DENV used for this manuscript, I suggest that the authors show/write down all IC50</p>	<p>Thank you for the suggestion. We have modified the manuscript accordingly.</p>	

	<p>results from curcumin and 6-gingerol, respectively.</p> <p>The author can also identify which serotypes (for each compound) that was affected the most.</p> <p>For example in 6-gingerol, DENV-2 was affected the most than the other three (put the statistical significance if there is any).</p>		
13.	<p>Results:</p> <p>Have the authors performed the statistical analysis to confirm the significance of their respective values? If not, I think it is necessary to perform that, so that we have more confidence in writing these sentences</p>	<p>Yes, we have added the statistical analysis values in the Results section.</p>	
14.	<p>Discussion:</p> <p>Could the authors please add more discussion regarding the results? For example, why does Curcumin have more significant antiviral effect on DENV-4? I believe it will add more value to this manuscript.</p>	<p>We could not answer the question why curcumin have more antiviral activity compared to other serotypes as this was not in our scope of research aims at the current study. More in-depth study is needed to answer the question. Nevertheless, we have included some possible reported mechanisms of Curcumin's antiviral properties i.e membrane-disturbing properties (Chen et al. 2013), altering membrane fluidity (Anggakusuma et al. 2014) and inhibiting cell binding (Mounce et al. 2017) (Discussion section). We have add a paragraph describing this limitation. We are confident that our results is of merit since revealing that DENV antiviral assay of compound need to be performed for all four serotypes.</p>	<p>Line 214-217 Line 232-236</p>
15.	<p>References:</p> <p>The authors are required to meticulously check the references before submitting. Some journals even reject the manuscript directly if the references were not fit to</p>	<p>We have re-checked each reference entry and use journal's style in our reference manager. We have made adjustments to specific points (e.g. scientific names, journal abbreviations, titles, etc) in the references section.</p>	<p>References section</p>

<p>their requirements. In this manuscript, even though it was automated using the software, the authors are still needed to double- or even triple-check them. For example: all scientific names (at least for plant to my knowledge) must be in italic.</p>		
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Reviewer B

No.	Comments	Reply action taken	Line number
1.	General comment: This research manuscript requires improvement according to comments	Thank you. We have made substantial revision based on the reviewers comments that hopefully can improve our manuscript.	Throughout the manuscript
2.	<p>Comment on Abstract: Abstract can not explain the activity of the test compound against the four types of denv serotypes.</p> <p>Which denv? It should not the mean and stdev for al denv, because I think the superiority of this study compared to others is using 4 denv serotypes.</p>	The abstract section has been modified to clearly explain the DENV serotypes. The description of mean values was changed to describe activity of test compounds of each DENV serotype.	Line 10 - 16
3.	<p>Comment on Introduction: Some references need to be updated. It is also necessary to pay attention to the use of scientific words.</p> <p>Maybe you can add why it requires the use of 4 kinds of denv serotypes It is also used to show the novelty of the research.</p>	<p>We have update the references and revise the scientific words accordingly.</p> <p>The use of four DENV serotypes has been highlighted to show the novelty of the study.</p>	<p>Introduction section Line 26</p> <p>Line 60-64</p>
4.	Comment on Materials and Methods:	Additional reference has been cited.	Line 88

	<p>Additional reference citations are required</p> <p>Cytotoxic test only to A549 cell, why not also done against DENV</p>	<p>Cell viability testing was done only for Curcumin and 6-Gingerol compounds since the aim of this assay was to test compounds in sub-cytotoxic level.</p>	
5.	<p>Comment on Results and Discussion:</p> <p>1. Data on cell viability should be displayed.</p> <p>2. Anova analysis between groups has not been seen. We recommend that these results be presented to see the conclusion that there is a significant difference in activity between doses.</p> <p>3. a. Statistical data analysis should be improved.</p> <p>b. Additional discussion is required according to comments.</p> <p>4. Why calculated mean and stdev compound activity against 4 types of denv? Does this study actually look at the differences in compound activity between the 4 denv?</p>	<p>1. We have added Figure 1 to show the effect of different concentrations of curcumin and 6-Gingerol to A549 cells.</p> <p>2. We have added statistical values measured using ANOVA statistics of each compounds groups for each DENV-serotype to describe the significant reduction of virus titer after compound treatment.</p> <p>3. Results section:</p> <p>a. Marker of significance (statistic <i>p</i> values) has been added in the Figures to better describe the data written in the Results section.</p> <p>b. Additional discussion has been added</p> <p>4. We have modified Table 1 and delete the mean and STDEV calculation. This study is descriptive only to look at antiviral effect of four DENV serotypes. Thank you for this significant comment.</p>	<p>Figure 1</p> <p>Figure 2 and 3</p> <p>Line 148 – 157</p> <p>Table 1</p>
6.	<p>Comment on Conclusions:</p> <p>Less sharp conclusions</p>	<p>We have re-written the conclusion for not being too sharp</p>	<p>Line 241 – 245</p>
7.	<p>Comment on Figures:</p> <p>Statistical analysis needs to be described more clearly</p>	<p>Description on statistical analysis performed has been added in the methods and results sections.</p>	<p>Methods and Results sections</p>

Reviewer C

No.	Comments	Reply action taken	Line number
1.	<p>General comment: This study uses is interesting, presenting natural based-compounds to determine their antiviral activities against DNEV. However the provided data in this study is still limited, thus it needs more detail discussion to make conclusions that fit the aim of this study.</p>	<p>Thank you. We have added new data in the Results section and performed statistical analysis to justify our results and make conclusions fit the study aim.</p>	<p>Throughout the manuscript</p>
2.	<p>Comment on Introduction: The authors need to explain the novelty of the study (e.g: the utilization of A549 cells, so it needs to be mentioned in the introduction, as well as the logic reason why using A549 cells instead of other cell lines), as curcumin has already been explored as antiviral for DENV in previous publications.</p>	<p>We have described the rationale for using the A549 cells in the Introduction section. Curcumin has been explored in previous publications but not on four DENV serotypes. This, we think, is the merit of our study.</p>	<p>Line 57-60</p>
3.	<p>Comment on Materials and Methods:</p> <p>a. The authors need to describe the curcumin that used in this study, why the purity of curcumin was pretty low (65%), the purpose of using Vero cells, and also reference for the utilization of BHK21 cells.</p> <p>b. The calculation of CC50 and IC50 also need to be explained in detail.</p>	<p>a. We were using a commercially available Curcumin from Sigma. The powder format has lower purity as determined by HPLC. This has been included as study limitation. Vero cells used only for isolation and propagating the DENVs stock as the commonly used cell line for this purpose. A reference for the use of BHK21 has been added.</p> <p>b. We have revealed the calculation of CC₅₀ which included 5 difference</p>	<p>Line 232 – 236</p> <p>Figure 1</p>

	<p>Why only used 3 measurement points? Also it might be suitable to use Anova instead of T-test since the groups were more than 2.</p>	<p>concentrations (Figure 1A and B). The measurement of IC50 was done in sub-cytotoxic concentration and 3 concentrations were selected. ANOVA statistics were used to calculate significance. We have discarded information on t-Test.</p>	<p>Line 124 – 125</p>
<p>4.</p>	<p>Comment on Results and Discussion: a. The results of the cytotoxic test should be presented to demonstrate the validity of the data. b. The data analysis for table 1 needs to check and revise. The authors should analyse the variance of each group and the differences between groups using Anova. The variance of the measurement results for each treatment was not shown yet. c. Each figure or graphic should be described for its replication treatment, statistical tests used, and the level of significance. d. The discussion should be directed at the validity of the methods, the significance of the results, and how the authors compare with the results that have been carried out using different cell models. The discussion should also mention the prospects of this study by looking at the limitation of this study.</p>	<p>a. We have added Figure 1A and B to depict the result of viability test of compounds. b. Thank you for bringing this up. Table 1 has been re-checked and calculation has been cross-checked with new software. The revised Table 1 is now contains updated numbers. In addition, ANOVA statistical significance results were added in the Result section and Figures 2 and 3. c. We have updated the Figures. d. We tried not to exhaustively discuss on possible mechanisms that cause the results and stick to being descriptive of the results. However, we have compared our results with other reports using only one DENV serotype. To date and to the best of our knowledge, this is the first study describing the antiviral effect of Curcumin and 6-Gingerol to all four DENV serotypes. Nevertheless, limitations to the study have been added.</p>	<p>Figure 1 Table 1 Line 136 – 157 Figures 1 – 3 Discussion section Line 232 - 236</p>

5.	Comment on Conclusions: The authors need to make conclusion based on the purpose and the discussion to the study	We have modified the conclusions to better answer the research aim/purpose, also based on comments from other reviewers.	Line 241 – 245
6.	Comment on References: The authors need to add several prior studies related to curcumin and DENV	Related references have been added.	References section
7.	Comment on Figures: As describe at the result comment	N/A	
8.	Comment on Tables: As describe at the result comment	N/A	