Author's response to reviews

Title: Evaluation of direct antiviral activity of Deva-5 herb formulation and extracts of five Asian plants against Influenza A Virus H3N8

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Author's response to reviews: see over
Dear Victor Kuete,

We are grateful to all the reviewers for their critical comments that help us to improve the manuscript. We corrected, where it was appropriate, the manuscript according to comments of the reviewers. Please find our detailed reply below.

**Reviewer 1 (Jianxin Chen):** “After reading the revision the manuscript no. 4774900231141904, I still insist that this manuscript provided very limited valuable information and is not suitable for publication. For assaying the antiviral properties of DEVA-5 and its components, the author determined the viral titer reduction after 30 min incubation in the presence and absence of the extracts of five plants and DEVA-5 in various concentrations. The authors claimed that their results suggested that *H. erectum, T. chebula* and *M. cochinchinensis* possess direct antiviral activities at dilutions from 1:3 to 1:14. But this direct in vitro antiviral activities don’t mean that they have antiviral effects in vivo. As we know, a number of compounds existing in many plants such as polyphenols have considerable antiviral activities in vitro, but have no effects in vivo. These compounds inhibit viruses usually by a nonspecific but not specific pathway. The authors did not provide any results to testify specific inhibition of the three plants extract on influenza virus. So I can’t agree the author’s conclusion that *H. erectum, T. chebula* and *M. cochinchinensis* could be promising sources of new antiviral drugs.”

**Authors’ reply:** We are grateful to Dr. Chen for valuable discussion. As far as we understood, Reviewer 1 has two main concerns about our results. Firstly, Dr. Chen notes that the antiviral action may be nonspecific. We addressed partially the question of specificity of action in “Discussion” section, when it appeared that extract of *Terminalia chebula* inhibit AIV H3N8, however, against AIV H1N1, no direct antiviral activity of water extracts of *T. chebula* was detected in the study by Badmaev et al., 2000. Thus, at least one of studied plant extracts possibly exhibit specific antiviral effect. From the other side, the broad specificity of antiviral action may be the benefit for practical approach. Indeed, broadly specific antiviral drug might be more convenient as medication than dozens of subtype-specific drugs against each of known AIV subtype.

The second concern is the ability of the studied extracts or their compounds to protect mammals in-vivo. The test of protective activity in-vivo includes extensive experiments with laboratory animals. This requires the strong ethical rationale for research to ensure that animal lives and research funds will not be spoiled without any results. Thus, at the first stage it is important to evaluate whether the studied formulation contains any antiviral substances at all or not. The approach when experiments in cell culture are done before the tests in laboratory animals is widely used in related field and are extensively published. We think that our results contain reliable, new and valuable information about antiviral properties of certain herb extracts. Therefore we believe that obtained results should be published to make these data available to scientific community involved in the search of novel antivirals derived from nature-borne substances. This would greatly stimulate further research of antiviral and bioactive potential of DEVA-5 and its components including in-vivo tests.

**Reviewer 3 (Sandra Adams):**

“Minor Essential Revisions
There are multiple instances where commas are used and there should be periods. for example:
1. In the Results Section, final sentence above "Virus Neutralisation by plant extracts" please correct: 0,13% and 0,03% (should have periods instead of commas).
2. In the Results Section, "Virus Neutralisation by plant extracts": (R>0,7 at p=0,05) should have periods instead of commas)
3. Figure 3 legend: \( \sim 1 \times 10^5 \text{PFU} \) (superscript 5)

**Authors’ reply:** We are grateful to Dr. Sandra Addams for careful review. Text is revised according to the reviewer’s suggestions; other analogous errors that were detected in manuscript are corrected as well.

**Reviewer 4 (Maja Nowakowski):**

“1. The authors clearly define the question addressed by their experiments testing the direct antiviral activity of a traditional compound Deva-5 and extracts of Deva-5 components. This does not include the molecular and phylogenetic analysis of the virus strain used, H3N8. Therefore, it is still suggested as Minor Essential Revision that the authors include in the discussion a statement they made in response to this reviewer’s comments: “The virus strain used in our study was previously characterized in very restricted set of experiments. Therefore, we sequenced the HA and NA genes to exclude possible misidentification of virus subtype or misplacement of virus stock. The genetic identification of virus subtype was performed by phylogenetic analysis in comparison with reference strains of each subtype”. This would adequately address our earlier concern: “A major concern is the considerable effort and space devoted in this work to the molecular and phylogenetic analysis of the virus strain because the data obtained are not discussed in relation to the rest of the work and no rationale is given for performing this extensive analysis. This part of the manuscript should be more fully integrated and its relevance to the rest of the work should be clearly explained”.

**Authors’ reply:** We are grateful to Dr. Maja Nowakowski for careful review. Text is revised according to the reviewer’s suggestions. Following statement is included in discussion to address the reviewer’s concern: “In this study we tested the antiviral activities of the traditional drug Deva-5 and its components against avian influenza virus H3N8 in cell culture. The virus strain A/Teal/Tunka/7/2010 (H3N8) used in our study was previously characterized in very restricted set of experiments. Therefore, we sequenced the HA and NA genes to exclude possible misidentification of virus subtype or misplacement of virus stock. The genetic identification of virus subtype was performed by phylogenetic analysis in comparison with reference strains of each subtype and confirmed the virus identity.”