Reviewer's report

Title: Antiviral activity and possible mode of action of ellagic acid identified in Lagerstroemia speciosa leaves toward human rhinoviruses

Version: 1 Date: 10 March 2014

Reviewer: Jayesh Mudgal

Reviewer's report:

Review of the Manuscript A9RBCB7

Background

1. Introduction written is not up to date as far as the background of the plant is concerned. Authors should mention all the known major medicinal values of the plant from literatures and give the suitable references.

2. Line 73; add the reference for the antiviral activity of the met. extract of the plant.

3. Introduction should state why author selected this plant to explore against viral infection specifically. Justify with suitable references.

4. Line 73, it is stated that “a methanol extract from the leaves of Giant Crape-myrtle (called Banaba in the Phillippines), Lagerstroemia speciosa (L.) Pers. (Lythraceae), was shown to have good antiviral activity toward HRV-4.” When the activity is known, then what was the objective of the current work? It should be clearly discussed.

5. Line 77, authors stated the objective of the current study to be “an assessment is made of the cytotoxic and antiviral activities of ellagic acid (EA) that comprise L. speciosa leaves toward HeLa cells and three HRV serotypes (HRV-2, HRV-3, and HRV-4).” This needs to be corrected and rewritten accordingly.

6. Its too early to state the commercial value of ellagic acid / plant leaves, as there are no preclinical and clinical studies. Author should be cautious while using these statements.

Material and Methods

1. Need to be rewritten as there are many grammatical mistakes. This section does not meet the expectation of scientific paper writing. There are spelling mistakes also. Authors are requested to correct and highlight the corrections in the manuscript.

2. The sentences need to be reframed correctly. Below written are a few example of incorrect sentences framed in the manuscript:

   a. Line 100-102: “Commercially available pure EA (#95% purity) and SRB and antiviral agent ribavirin were purchased from Sigma-Aldrich (St. Louis, MO) and Tokyo Chemical Industry (Tokyo), respectively.”

   b. Line 102-104: Anitbiotic-antimycotic and minimum essential medium (MEM)
and fetal bovine serum were supplied by Invitrogen (Grand Island, NY) and PAA Laboratories (Etobicoke, Ontario, Canada), respectively.

3. Line 124, extraction and isolation procedure should be explained more clearly. Fractionation process can be explained using the chart or diagram to make it more precise and clear.

4. Out of 19 ethylacetate fractions, author used 13 and 15 as an active fraction. But, there are no data mentioned in the manuscript regarding the activity of the ethylacetate fractions. How the authors arrived at this conclusion?

5. HPLC chromatogram is must to substantiate the findings of isolation process and to characterize the phytoconstituent(s) present in the fractions. Authors are suggested to include the chromatogram of methanolic extract and the isolated fractions.


7. Line 187, Infectivity of HRV particles: Its is stated that HRV was preincubated with the test compounds and after ensuring the removal of unbound virus, again the test compounds were added to the cell culture medium. What is the justification of this type of treatment design? This suggests the double exposure of the viruses with the treatment. Is it conventional?

8. Why in all the study design, author did not include the active fraction and compared with that of EA and Ribavirin. Whether active fraction possesses only EA?

9. There are inconsistencies in the HRVs against which the test compounds were assessed. Specifically, antiviral activity was assessed against all three HRVs, however, other test were performed using HRV-4 only. What was the rationale behind such experimental design?

Results:

1. HPLCchromatogram of ethylacetate fraction and the extract must be included in the manuscript for the identification of active principle(s), with respect to standards used.

Discussion

1. The possible mode of action, by inhibition of RNA replication has been proposed as the mechanism of action of EA. Author should discuss the mechanism and correlate with the functional group present in the active principle (with citing the suitable references).

2. Why authors focused only upon methanolic extract only? Authors should give the justification in the discussion.

3. In the current work, author claims for the presence of Ellagic acid in the EtOAc fraction of same plant leaves methanolic extract. However, H.J. Choi et al (2010) investigated Banaba leaves extract previously for its antiviral activity. It is interesting to see that Ethyl acetate fractionation yielded an active principle orobol 7-O-d-glucoside (O7G). O7G found to be much more efficacious as compared to Ellagic acid in the current study against HRV strains. Author should
highlight these finding in the discussion.
4. IC50 / dose of the pure compounds should be converted in \( \mu \text{M} \) instead of \( \mu \text{g/ml} \).

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, and I have assessed the statistics in my report.