Reviewer's report

**Title:** In vitro anti-Herpes simplex virus activity of crude extract of the roots of Nauclea latifolia Smith (Rubiaceae)

**Version:** 1  **Date:** 8 August 2013

**Reviewer:** Keivan Zandi

**Reviewer's report:**

Authors conducted a suitable research in the field of antiviral research and herbal medicine but they need to do following corrections and clarifications to make their manuscript in an excellent format.

**Major Compulsory Revisions:**

**Abstract**

1- In the last line of Background part of the Abstract, authors mentioned about the impact of HSV-2 in HIV transmission. However, this statement in the present format is not accurate as it could be confusing for the readers as there is no real relationship between HSV-2 and HIV incidence. I agreed that because HSV-2 is more prevalent between sex workers or people who does not have safe and protected safe and on the other hand the incidence of HIV is also among the same population is higher than the others BUT it is not because of relationship between biology of those viruses so it is strongly recommended to remove that sentence.

2- The methods part, under Abstract needs to be rewrite because of some grammatical errors.

3- Statistical program name is GraphPad PRISM not PRISM alone. The other question is: why they used version 4 as now a days version 6 is available.

**Background:**

1- Page 4: Name of the antiviral drugs should not be started with capital letter.

2- Why there is no citation to the other available herbal medications for HSV such as, Aloe vera or red algae creams or gels? there are too many approved and studied herbal medications for HSV infections. Therefore, authors must include some studies related to those plants as well not just African plants.

**Methods:**

1- Authors must cite to the method which they used for development of drug resistant strain of HSV-2.

2- Is heparin the only reagent that authors used? If no please include all reagents under the same subheading.

3- HSV Inhibition Assay is not a correct subheading so it must change to the proper term.

4- The MOI is different from pfu but authors mixed them together so it must be
corrected.

5- Why authors used just one concentration for virucidal activity?

6- Attachment assay is somehow similar to the virucidal assay. It would not recommended to pre-treat the virus itself with the extract for attachment assay. On the other hand after 4 degrees incubation they MUST allow virus to enter to the cells for 1 h at 37 degrees with medium not with CMC containing medium. The method in this part is not acceptable.

7- In entry assay, authors allowed the viruses for entry for 3 h, but for post adsorption assay they adjust the whole process for adsorption and entry for 2 h. It is not consistent.

Results:

1- Authors must mention the exact percentage of DMSO. In the present format of the manuscript they wrote, below 1%!!! it could be 0.8% or 0.5% or,?,? It must be clear.

2- On page 11, authors mentioned that no extracts' concentration shows cytotoxicity against Vero cells then they wrote that CC50 is above 100 microgram per ml so how it could be concluded??

3- If the exact CC50 was not calculated the how they calculated SI as SI can be calculated based on defined IC50 and CC50.

4- Authors have used 33 microgram per ml for virucidal activity and on the other hand they mentioned that they diluted the mixture of virus and extract at the time of cell infection below than the concentration of the extract which showed antiviral activity BUT they did not mention about the results. It is totally unclear and it is not acceptable in this format.

5- There is a major problem in this manuscript, authors mentioned in the first part of the antiviral assays in the method under subheading: HSV inhibition assay that they did the treatment starting from the time of virus adsorption and even after adsorption continuously BUT there is no available data for that treatment in result part!!!! on the other hand they just mentioned that post adsorption treatment gave them the best result so what about the continuous treatment that they mentioned earlier.

6- They did not mention about any difference or similarities of their results for sensitive or drug resistant HSV-2 strains.

Level of interest: An article whose findings are important to those with closely related research interests

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

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

Declaration of competing interests:
I have no competing interests.