Reviewer's report

**Title:** Histone deacetylase (HDAC) inhibitory and Antiproliferative activities of phenolic-rich extracts derived from the rhizome of Hydnophytum formicarum Jack.: Sinapinic acid acts as HDAC inhibitor

**Version:** 2  **Date:** 3 July 2013

**Reviewer:** Leah Cosgrove

Reviewer's report:

Review of Manuscript titled: Histone deacetylase (HDAC) inhibitory and Antiproliferative activities of phenolic-rich extracts derived from the rhizome of Hydnophytum formicarum Jack.: Sinapinic acid acts as HDAC inhibitor

http://www.biomedcentral.com/imedia/2524370479290375_article.pdf

1. Is the question posed by the authors well defined?
The main premise that the authors in this manuscript propose is that the traditional medicinal herb (rhizome of Hydnophytum formicarum Jack) has anti-cancer properties due to its histone deacetylase (HDAC) inhibitory activity. They isolated ethanolic and phenolic extracts from this herb and showed by HPLC that the main compound responsible for this activity is sinapinic acid. They then compared the extracts derived from this herb and sinapinic acid with butyrate a well known HDAC inhibitor with anti-proliferative activity on 5 cancer cell lines. They showed a differential response to inhibition of proliferation or induction of apoptosis was cell line dependent and did not appear to correlate with HDAC activity.

2. Are the methods appropriate and well described?
The methodology used by these authors is appropriate for this type of study.

3. Are the data sound?
The data appears robust but there are some specific issues that are not clear regarding the extracts. Did they compare DMSO, ethanol, methanol, ethyl acetate, and hexane to see the effects by the carrier alone or at different concentrations, as these solvents will effect viability of cells? As in the anti-proliferative assay they appeared to only compare with 0.05% DMSO or H2O alone. Also how did they determine the residual solvent concentration for their fractions so they could compare the effect of carrier solvent alone?

The other issue is with the HDAC inhibition. The authors show gel photos but how did they quantitate this? It would have been better to scan the actual gels and compare with total histone. Also figure 4 shows a representative gel, how many biological replicates were done? The other issue with figure 4B it is difficult to draw conclusions to how histones are being effected and to also what cell line they are looking at. Are the results the same for the other 4 cell lines?

Identification of one of the active components of phenolic-rich extract of H.
formicarum Jack Rhizome was determined to be sinapinic acid based on identical retention time and spectra of known phenolic standards under the same chromatographic conditions. Are the authors sure that no other components could run at the same retention time or spectra?

4. Does the manuscript adhere to the relevant standards for reporting and data deposition? Yes, it does.

5. Are the discussion and conclusions well balanced and adequately supported by the data? Yes.

6. Are limitations of the work clearly stated? No, not really. With the different extracts how did the authors account for how much phenolic activity was being derived and was this reproducible on different extractions? It is unclear if they did the extraction only once. Also there is the issue regarding the gels as discussed in question 2 in that the gels were not scanned so no statistics could be done to see how reproducible their findings were.

7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished? Yes

8. Do the title and abstract accurately convey what has been found? Yes

9. Is the writing acceptable? Yes, I have noted grammatical errors and they are minor.

   • Discretionary Revisions (which are recommendations for improvement but which the author can choose to ignore): Nil
   • Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
     • Page 2, Line 15 & 16: effective should be effectively
     • Page 12, line 6: “acid inhibited HDAC activity not only in vitro but also in the cells” Cells are in vitro. This should be corrected.
     • Page 14: Line 21,22The authors say “Based on our findings that sinapinic acid possesses antiproliferative activity more effective than a well-known HDAC inhibitor sodium butyrate”. In their figure 1 this is not the case for all of the 5 cells they examined. For Hela and HT29 cells this is true but not for HCT116 and Jurkat cell lines were they see the reverse. This should be corrected.
   • Major Compulsory Revisions (which the author must respond to before a decision on publication can be reached) Nil

**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: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
'I declare that I have no competing interests'