Reviewer's report

Title: A phenolic ester from Aglaia loheri leaves reveals cytotoxicity towards sensitive and multidrug-resistant cancer cells

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Reviewer: Vivian M. Rumjanek

Reviewer's report:

Major compulsory revision-

1- To be able to distinguish between non-specific cytotoxicity and an anti-tumor effect, it is necessary to perform a control using normal cells. It would be important to use normal peripheral blood mononuclear cells activated with a mitogen such PHA to see if the effect is specific towards tumor cells.

- As it is stated below the question of the need for a normal control has not been dealt in a satisfactory manner.

- Despite what the authors said, this first part is not in page 16 of the revised manuscript.

"When Maldi531.2[M+H]+ was assayed against non-cancer cell line (AA8) no toxicity was observed. When crude extract of A. loheri which yielded Maldi 531.2[M+H]+ was tested against AA8 no toxicity was observed. It is to be noted that A. loheri is edible and used by indigenous people of Bataan, Philippines as staple vegetable. AA8 is used in many studies as a basis to compare performance of cytotoxic samples between cancer and non-cancer cells. Still, AA8 is a murine cell line (as it is from Chinese hamster ovary)"

- I could not find the results using AA8. Has this been published? If not the data needs to be shown in the present paper so that the concentrations tested could be compared.

AA8 is an epithelial-like cell line and obtained from hamster (not a mouse, therefore it not murine). Anyway, it would be good to have controls in this manuscript.

"; it is recommended that these results of Maldi531.2[M+H]+ against non-cancer cells be tested with non-cancer, human cell lines such as normal peripheral blood mononuclear cells."

- Exactly, because of that (being a leukemia the best control is normal activated PBMC) we expect this experiment to be performed.

-The following part has been added by the authors to the manuscript:
"Although it is important that the cytotoxic agent be determined to be specific only to cancer cells, it is an established fact that many of the presently used cancer chemotherapeutic agents are not specific against tumor/malignant cells only. They also affect fast dividing normal cells such as those in the blood, in the digestive tract and other organs leading to a number of side effects like vomiting, hair loss, and the like. Hence, a number of studies that seek to mitigate these effects on normal cells such as on drug delivery specifically targeting cancer cells are being actively explored. We can also suggest this as an offshoot of this study as part of the recommendation or future directions/follow up studies."

- Even if most cytotoxic drugs are somewhat toxic to normal cells, it is fundamental to know what it is the therapeutic window. Of course, non-specific cytotoxicity is undesirable for a compound and, to be dealt with, it is necessary to have information on the toxicity levels.

The authors provided the following answer to my question related to apoptosis and dosage:

"On the issue of using unrelated chemotherapeutic drug in the experiment, I used 10µM camptothecin alongside with 10µg/mL doxorubicin as one of my positive controls.

Considering the IC50 values of Maldi 531.2[M+H]+ in HCT116 (data not shown), CCRF-CEM and CEM/ADR5000 which are 3.9µg/mL, 0.02µM and 0.03µM respectively, the isolate could be considered a potential cytotoxic agent against cancer cells.

Concerning the right concentration to consider a compound as an anticancer drug: there is not fixed concentration. However, in our own investigations, we use 10 µg/ml as cutoff (Efferth et al., Molecular Cancer Therapeutics, 2008;7:152-161)."

- This point has been raised regarding the apoptotic index. If you state that according to MTT the IC50 for leukemic cells is 0.02uM-0.03uM, it is surprising that 0.05uM has no effect and 0.5uM induces a maximum apoptosis of 5% of the cells. When was the assay performed? At 24h?

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

**Quality of written English:** Acceptable

**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