Author’s response to reviews

Title: Anti-inflammatory and cytotoxic evaluation of extracts from the flowering stage of Celosia argentea

Authors:

Oluwafunmilayo Dorcas Adegbaju (funmaj2005@gmail.com)

Gloria Aderonke Otunola (gotunola@ufh.ac.za)

Anthony Jide Afolayan (aafolayan@ufh.ac.za)

Version: 3 Date: 17 Feb 2020

Author’s response to reviews:

Response to Reviewers’ Comments BCAM-D-19-000583
Reviewer reports:

Guan-Jhong Huang (Reviewer 1): Anti-inflammatory and cytotoxic activities are closely linked to plant components. Thus, HPLC must be used to identify compounds of different extracted Celosia flowering stages.
Response: Inasmuch as the authors would have loved to do a HPLC analysis of the extract, it is not possible with this present submission. However, this assay along with other isolation and characterization assays are the next stage of the study.

Reviewer 2 (Reviewer 2): PEER REVIEWER ASSESSMENTS:

OBJECTIVE - Full research articles: is there a clear objective that addresses one or several testable research questions? (Brief or other article types: is there a clear objective?)
Yes - there is a clear objective

DESIGN - Is the current approach (including controls and analysis protocols) appropriate for the objective?
Yes - the approach is appropriate

EXECUTION - Are the experiments and analyses performed with sufficient technical rigor to allow confidence in the results?
No - there are minor issues

STATISTICS - Is the use of statistics in the manuscript appropriate?
Yes - appropriate statistical analyses have been used in the study
INTERPRETATION - Is the current interpretation/discussion of the results reasonable and not overstated?
No - there are minor issues

OVERALL MANUSCRIPT POTENTIAL - Has the author addressed your concerns sufficiently for you to now recommend the work as a technically sound contribution? If not, can further revisions be made to make the work technically sound?
Probably - with minor revisions

PEER REVIEWER COMMENTS:

GENERAL COMMENTS: Experimental design- The experiments lack important controls. For example, the authors used 3 extraction methods (acetone, water, and methanol), which show differential effects. However, because the authors did not show (or test?) vehicle alone (extraction buffer), it is unclear whether differences are due to the extraction buffer, or the compounds within in the extraction buffer.

Response: Response: after solvent extraction of the samples, the solvents were removed using a rotavapor and dried extracts (devoid of extraction solvents) were recovered (Line 114-115). Prior to the main assay, the extracts were reconstituted in a final DMSO concentration of 0.2% in the highest sample concentration. However, the same final DMSO (vehicle) concentration was tested (not reported) on each of the assay to eliminate solvent/vehicle interference. The outcome shows no interference by the vehicle whatsoever on the assay (Material and methods)
The authors also included some controls (silymarin and melphalan), but did not discuss why there were used, and did not include a vehicle only control for these compounds. Again, it is unclear whether the changes are due to the compound of the diluent.
Response: Silymarin and Melphalan were used as POSITIVE controls. As stated above, a trial assay of the diluent (not reported) was done to eradicate the possible interaction of the diluent on the assay.

Reviewer's comment: **I am happy to see that the authors did test a DMSO control. This is an important control. I don't understand why they chose not to report this finding.
*Response: The findings of the DMSO TEST has been included in the graphs section as figure 1.

Response: Silymarin was used as a positive control for the anti-inflammatory assay. This was mentioned in the manuscript on page 6, line 132, under the METHODS section. “Melphalan was used as a positive control for the cytotoxicity assay” This sentence has been added to the manuscript and can be found under the METHODS sections on page 7, line 158.

The authors chose to measure NO as a measure of inflammation. There are many markers or inflammation, and there is no discussion on why NO was specifically chosen. I also don't understand why the authors chose murine preadipocytes for the cell viability assays.
Response: Explanation for using NO as a marker of inflammation has been given (line 196-210). *this is sufficient*

Reason for the choice of 3T3-L1 murine preadipocyte cell line used for this assay has been explained (line 201). *also sufficient*

Execution- The data are presented with error bars, so I presume the authors ran the experiments in duplicate or triplicate, but I cannot assess. Oddly, the results are presented as "1st and 2nd trial". The results of the two trials are somewhat inconsistent. This is not surprising given the nature of the experiments; however, more replicates should be done in order to make more concrete conclusions.

Response: Each experiment was replicated four times (indicated in the figure legends as n=4). The 1st and 2nd trials represent different planting seasons and not replications of the same samples. *This makes good sense. I still cannot find in the manuscript where it mentions that the 1st and 2nd trial are different planting seasons. This would help the reader if you made it more clear.*

Response: The explanation concerning the first and second trial as two different planting season has been stated under the Methods section, on line 106.

Statistics- The statistics as described seem appropriate. The figures are not labelled well; they are very busy and hard to read.

Response: the comparison was done with response to the untreated control. Hence, the '*' and '#' indicates NO significance and significantly different from the untreated control respectively.

Interpretation- I am not convinced of the increase in cellular proliferation that you report. More studies should be done to explore this phenomenon. The fact that you used these data to say that the extracts can be used for wounds, etc is a stretch. The authors also conclude that "all extracts" are "not toxic" and that "C. argentea could be safely used as an anti-inflammatory with no-side effects". The data do not support these conclusions.

Response: The relevant sections have been revised. Where necessary, phrases have been changed to convey the correct meaning (Lines 216-220; 227-229). *This reads much better*

ADDITIONAL REQUESTS/SUGGESTIONS:

I would strongly consider 1) assessing multiple inflammatory markers and 2) conducting additional toxicity assays in multiple cells lines. *Response: The reviewer’s suggestions are greatly appreciated and noted for future studies.

The authors also need to reasonably state conclusions from the data.

Response: Conclusion from data has been rephrased to avoid overstating the results (Line 238 to 240). It now reads: The observed anti-inflammatory action agrees with the reported traditional
use of C. argentea for the treatment of inflammation. Although it is exciting to note that the aqueous and methanol extracts are non-toxic, further in vivo tests are recommended to validate this outcome.

*Lines 231-234 are still overstated. You cannot make this conclusion from your evidence presented*
Response: Conclusion from data has been rephrased to avoid overstating the results (230 to 240)

REQUESTED REVISIONS:
Overall, the authors did a good job addressing concerns. I would still like them to include the DMSO control.
*Response: DMSO control has been included in the graph section

I would also like them to clarify that trial #1 and trial #2 were different planting seasons (they did this in the letter but I cannot find this in the manuscript).
Response: This has been added to the manuscript (line 106)

They still overstate their results "The non-toxic potential of all the extracts in this study corroborates the traditional use of the plant as vegetable and medicine; and indicates that C. argentea could be safely used as an anti-inflammatory agent with no side-effects." The compound was non-toxic in their experiments, but this does not mean it will be non-toxic without side effects in vivo.

Response: Conclusion from data has been rephrased to avoid overstating the results (Line 238 to 240. Please refer to the newest comment on this section).