Author’s response to reviews

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

Authors:

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Author’s response to reviews:

Reviewer 1
Comments for the Attention of the Author(s)

In this manuscript, the author’s reports “Anti-inflammatory and cytotoxic evaluation of extracts from the flowering stage of Celosia argentea”. This article focuses on the traditional use of C. argentea for painful inflammatory conditions and encourages its possible use as lead for the development of novel, non-toxic, anti-inflammatory agents. The paper is presented in detail, and the results are carefully discussed.

Major comments:
There are some points should be checked are followings:

1. HPLC must be used to identify compounds of different extracted Celosia flowering stages.
Response: This is noted and will be considered for the next phase of study. However, for this study, the assay was on the crude extract of the flowering stages as shown on the title.

2. The authors should show the mechanism of the Anti-inflammatory and cytotoxic activity of the flowering stage of Celosia argentea.
Response: The possible mechanism of the anti-inflammatory activity of the flowering stage of C. argentea has been included in the manuscript (Line 210-220).

3. In the results and discussion: the author should strengthen the results of the research in the field of mechanisms.
Response: Appropriate controls were used in this study; Silymarin was used as a standard control to check for cell viability while Melphalan was used as standard control in the cytotoxicity assay. Furthermore, results have been rephrased in areas where it was perceived as being overstated for clearer outstanding (Lines 216-220; 227-229)
* What is your overall impression of the study? The study is designed to test the possible anti-inflammatory properties of multiple extracts of C. argentea. The authors also examine potential cytotoxicity of these extracts. This study was done using cell culture methods, which is completely appropriate at this stage.

* What have the authors done well? I appreciate that the authors are looking at both therapeutic effects (anti-inflammatory effects) as well as potential toxic effects (cell viability). It's important to appreciate both at an early stage.

* In what ways does it not meet best practice? The experiments in the paper contain significant variability and do not include appropriate controls. Furthermore, the authors overstate their results in a few areas.
Response: Appropriate controls were used in this study; Silymarin was used as a standard control to check for cell viability while Melphalan was used as standard control in the cytotoxicity assay. Furthermore, results have been rephrased in areas where it was perceived as being overstated for clearer outstanding (Lines 216-220; 227-229)

REQUESTED REVISIONS:

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.

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

Statistics- The statistics as described seem appropriate. The figures are not labeled 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).

ADDITIONAL REQUESTS/SUGGESTIONS:

I would strongly consider 1) assessing multiple inflammatory markers and 2) conducting additional toxicity assays in multiple cells lines. The authors also need to reasonably state conclusions from the data.
Response: 1) This suggestion is noted for further studies. 2) However, Brine Shrimp lethality (toxicity assay) test had been done on the test sample and published by the authors (See ref: Adegbaju, O. D., Otunola, G. A., & Afolayan, A. J. (2019). Research Article Influence of Plant Maturity on Antimicrobial Properties and Toxicity of Celosia argentea.). Conclusion has been rephrased and properly stated from the data (Line 238-240).