Author’s response to reviews

Title: Potential in vitro anti-allergic, anti-inflammatory and cytotoxic activities of ethanolic extract of Baliospermum montanum root, its major components and a validated HPLC method

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Author’s response to reviews:

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Dear Editor-in-Chief

BMC Complementary and Alternative Medicine

Manuscript ID: BCAM-D-18-01511 entitled ‘Anti-allergic, anti-inflammatory and cytotoxic activities of ethanolic extract of Baliospermum montanum Root, its major components and a validated HPLC method’

Thank you very much for your letter dated December-7, 2018. We have revised the manuscript based on the reviewers’ comments. The revised texts according to the comments are highlighted in yellow color. The grammatically errors edited by a native English speaker are marked in red alphabet. The author’s responses are detailed in the attached file.
We are agreed with all comments made by the reviewers. We would like to express our sincere thanks to all reviewers for their kind advices.

Don’t hesitate, if you need some information. However, my Ph.D student (Weerachai) need acceptance letter for finish Ph.D program within January. Please help him, if it is possible.

I hope that you will accept our manuscript to publish in your Journal. I and my Ph.D student are looking forward to see your mail soon.

Yours sincerely,

Arunporn Itharat, Ph.D

Corresponding Author

Author’s response to reviews

Manuscript ID: BCAM-D-18-01511

Anti-allergic, anti-inflammatory and cytotoxic activities of ethanolic extract of Baliospermum montanum Root, its major components and a validated HPLC method’

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Ifeoma C Ezenyi (Reviewer 1):

Thank you very much for the reviewer’s comments. We have revised the manuscript according to the comments. The revise texts of reviewer 1 is in yellow-highlight, of reviewer 2 is green-highlight. The grammatically errors of this manuscript were edited by native English speaker and are marked in red alphabet.

1. line 82: delete etc
   Author’s answers: Delete etc. After itching at line 81

2. line 87: replace interested activity with biological effects
   Author’s answers: Replace ‘interested activity’ with ‘biological effects’ [P.4, line 95-96]

3. Rephrase line 91-93
   Author’s answers: Rephrase these sentences of background. Line 85-105

4. line 98: ‘...hexsaminidase released from RBC-2h3 cells
   Author’s answers: Change to be ‘from RBL-2H3 cells’ [P.4, line 98]

5. ‘...effects of B. montanum crude extract...are also reported'
   Author’s answers: We rephrase the background section. [P.4-5, line 77-105]

6. line 104: specify part of B. montanum collected
   Author’s answers: All parts of plant were collected and the roots were separated later. [P.5, line 109]

7. line 158: state concentrations of serial dilutions
   Author’s answers: State the serial concentrations. [P.8, line 164]
8. line 167: formula for calculation of inhibition (5) not clear. The expression T.B.N. and C.B.N are not mathematical, use appropriate expression and symbols

Author’s answers: We are sorry about the symbolic error. They are ‘T – B – N’ and ‘C – B – N’.

[P.8, line 173]

9. line 178: state concentrations of serial dilutions

Author’s answers: State the serial concentrations. [P.9, line 186-187]

10. line 191-192: There are no results to show that cell viability was >70%, this should be included in table 2

Author’s answers: Add in table 2 already.

11. Line 212: Method of analysis should be shown in subsection on statistical analysis

Author’s answers: Delete this sentence and add to subsection on statistical analysis.

[P.13, line 286-287]

12. line 218: 'hexanes' should be corrected to 'hexane' throughout

Author’s answers: Change at line 228.

13. line 349: concentrations were used, not doses.

Author’s answers: Change the word ‘dose’ to ‘in direct proportion to their concentrations’ which following native English prove at line 356

14. line 348: Change 'liver cancerous cells' to 'cancerous liver cells'

Author’s answers: Change already at line 355
15. line 355: Rephrase this sentence as ...the cytotoxic effect of the extract and compounds against normal cells were lower compared to their effects against cancerous cells, showing their selective cytotoxic property.

Author’s answers: Rephrase the sentence according to reviewer’s comment at line 361-363

16. Figures 2 and 3 are unnecessary as the data has already been presented in tables 1 and 2

Author’s answers: Thanks for your suggestion, however, we would like to show the picture that present how differences between the tested sample and the positive control.

Sikiru Olaitan Balogun, PhD. (Reviewer 2):

In this manuscript titled Anti-allergic, anti-inflammatory and cytotoxic activities of ethanolic extract of Baliospermum montanum root, its major components and a validated HPLC method" the authors claimed to have investigated the anti-allergic, anti-inflammatory and cytotoxic activities of the crude ethanolic extract of the root of B. montanum. In addition, they also conducted isolation of major phytochemical components of the same. They also mentioned to have developed and validated HPLC method for the determination of the crude extract's major compounds.

The activities investigated were all in vitro related, and these include inhibitory effect of the crude extract on β-hexosaminidase released from RBL-2H3 cells, inhibition of nitric oxide (NO) production from RAW 264.7 cells and cytotoxic activity against cancerous liver cell lines (HepG2 and KKU M156) using sulforhodamine B (SRB) assay.

Although, the study is interesting, there are serious concerns to be addressed English language issues. The manuscript is filled with serious grammatical and spelling errors.

These make the reading and understanding if the manuscript very difficult. I suggest that the authors make use of the service of a native English speaker for English corrections.

Author’s response

Thank you very much for the reviewer’s comments. We have revised the manuscript according to the comments. The revise texts of reviewer 1 and 2 are in yellow and green-highlight respectively. The grammatically errors of this manuscript were edited by native English speaker and are marked in red alphabet.
Title

I believe the use of the terms are misleading, since only in vitro activities were studied and no in vivo effects were shown. It would therefore be out of place to state that the activities studied represented true anti-allergic, anti-inflammatory studies, since these are multifactorial in vivo conditions. I therefore suggest the title be changed to something like this "Potential in-vitro anti-allergy, anti-inflammatory, "

Author answer : Change the title to ‘Potential in-vitro anti-allergic, anti-inflammatory……..

Background

There is need to revise the way it has been written. For example in line 89 p 5, the sentence should begin by "Previous preliminary…" Anti-allergic activity As Passante et al (2009) concluded "While RBL-2H3 cells may be useful as a model for mast cell IgE-mediated degranulation, other aspects may not be representative and they may share similarities with basophils rather than with other histamine-releasing cell types." Based on this premise as well as others, it would have been better if the authors could include one or more in-vitro model or at best, one in vivo model of the disease in question. Anti-inflammatory activity This title may be changed to in vitro anti-inflammatory activity.

The authors should also carefully review the language errors in all the description of methods as well.

Author answer : Thank you for the advices. We rewrite the background according reviewer’s comments.

[P.4, line 85 – 105]

Results

Isolation and identification of pure compounds Perhaps the authors wish to state on page 14 line 287 (Additional File 1) as supplementary file. It is not clear why the authors said that it is unfortunate that a simple isolation method did not work.

Author answer :We discard this sentence for misunderstanding. My meaning is unfortunately because we have to also use semi-preparative HPLC for isolation so we discard this sentence.
Inhibitory activity of NO production from LPS-induced RAW 264.7

On page 16 L. 332 and 333, the authors stated that "High concentration of NO production from inducible NO synthase (iNOS) in macrophage causes several inflammatory diseases including rheumatoid arthritis and osteoarthritis". However, It is one thing to say that excessive NO production is linked to inflammation but it is wrong to claim that it causes inflammatory diseases, as stated in the manuscript. Besides, 50 µg/mL is way too high concentration for an in vitro study. What was the basis for selecting this concentration? Why was dose-response effect not considered?

Author answer : Thank you very much for the reviewer’s suggestions. We rephrase this sentence according to the reviewer’s comments.

[P.14-15, line 339-341]

Besides, 50 µg/mL is way too high concentration for an in vitro study. What was the basis for selecting this concentration? Why was dose-response effect not considered?

Author answer: We selected the concentration of 50 µg/mL for preliminary screening step. With this idea, we can picture how differences of %inhibitions between the tested sample and the positive control prior to investigation of IC50.

Why was dose-response effect not considered

Author answer: The concentration-response effects are shown in table 2.

Cytotoxic activity

What are the criteria for classifying a substance as having cytotoxic activity? The authors should state their criteria for doing so. The authors may consider the work by Suffness, M., Pezzuto, J., 1990. Assays related to cancer drug discovery.

Author answer: Thank you for the reviewer’s advice. We additionally stated the reviewer’s advice in the discussion section.[P.19, line 407-412]
The authors also indicated that the extract and the isolated compounds exhibited selected toxicities, however, selectivity index was not calculated.

Author answer: We calculate and add them in Table 3.

Discussion

The authors should focus the discussion squarely on their findings and relates these to what is in the literature. The authors made some conclusions, whereas the models used are different.

For example, they stated on page 19 L 392 -394 " The anti-allergy of the crude ethanolic extracts was potent similar to previous report of leaves of B.montanum which reported mast cell stabilization and anti-histamine in systemic anaphylaxis model in vivo. It would be wrong to state that it present 'potent' anti-allergy activity, at least mention should be made to the differences in the models used.

Author answer : Thank you, we fully agree with the reviewer.

We rewrite some sentences at line 400-401

We scope the discussion of our findings and related them to literatures.

We discard our conclusions that compared to different model.

The authors stated on p21 L 428 - 429 that "Due to few researches of propiophenones on biological activities, therefore, we compared the isolated propiophenones with phenylpropanoids which have analogue chemical structure and were widely". However, this doesn't seem to be correct, as analogues may possess widely different biological and pharmacological activities. If there are no studies, they should limit the discussion based on what they have. The authors also failed to discuss on the point why the crude extract demonstrated higher activity than the isolated compounds alone.

Author answer : Thank you very much for the suggestion. We would like to discuss the structure-activity relationship (SAR) of each activity we have tested. We would like to discuss what part of the chemical structure relating to their presented activities. However, we rephrase and shorten these sentences for more understanding. [P.20, line 434-447]

The authors also failed to discuss on the point why the crude extract demonstrated higher activity than the isolated compounds alone.

Author answer: Thanks for the reviewer’s advice. We additionally stated the suggestions relating to multiple components synergistic effects in the manuscript. [P.20, line 429-433]