Author's response to reviews

Title: Evaluation of Anthelmintic potential of the Ethiopian Medicinal Plant Embelia schimperi Vatke in vivo and in vitro against some Intestinal Parasites

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Author's response to reviews: see over
Dear Editor

We the authors of the manuscript entitled “Evaluation of Anthelmintic potential of the Ethiopian Medicinal Plant *Embelia schimperi* Vatke *in vivo* and *in vitro* against some Intestinal Parasites” with MS ID: 2079151901541793 have carefully reviewed and accommodated the comments suggested by the two reviewers. We have given responses to the issues addressed by the reviewers line by line.

We are ready to accommodate any further comments which might arise from the revised manuscript.

Warmest regards,

Yared Debebe
Reviewer: Adeyemi Oladapo Aremu

Line 118 Phytochemical screening

If this aspect of the study is required by the authors, reference must be included for the tests. There is no indication of the phytochemicals targeted by the authors. Please amend accordingly.

Response:

After a careful revision on the need of this section we decided to exclude the phytochemical screening part as it does not have a direct implication on the study.

Line 122: Isolation procedure

In the present form, I have serious concerns on the procedure outlined by the authors. It appears very superficial and I do know isolation procedure are often more intense and long. Authors need to clearly and meticulously present the isolation process used for the identified compounds. Also, authors must re-check their yields for correctness. Obtaining a pure compound (5 g) in 3 quick steps appears too simple and unrealistic. The purity of the isolated compound is missing and must be clearly stated. Please clarify what is meant by ‘hot’ ethyl-acetate and ‘cold’ hexane.

Response

The isolation procedure we followed might seems easy and unrealistic. But we have had series of laboratory experiments in optimizing the extraction condition of *E. schimperi* using various solvent systems. We have published an article with the title “Quantitative determination and optimization of extraction conditions for embelin in *Embelia schimperi* by UV-VIS Spectrometry” which explains every details.

We have also cited papers related to isolation and characterization of embelin from different species by some authors and included in the discussion part.

In this manuscript we cited the aforementioned article which we believe clarify the issues such as precision and accuracy.
Line 239: Discussion

The discussion is too brief and superficial. Discussion relating to the phytochemical and safety evaluation observed in the study is completely missing. Authors must remove these aspects from materials and methods as well as the result section if is not important to their study.

Response

Discussion related to safety evaluation has been added. More literatures have been consulted to make the discussion part more comprehensive.

Minor Revision

Abstract

Line 35: delete “Medicinal plants such as’

Response: Deleted

Line 39: Delete ‘generated to’ and change ‘evaluate’ to ‘evaluated’

Response: ‘generated to’- deleted; ‘evaluate’ changed to ‘evaluated’

Line 54: delete ‘indeed’

Response: ‘indeed’- deleted

Line 55-56: Rephrase sentence for clarity

Response:

The “Hence the findings of this study confirm the traditional usage of the plant to combat taeniasis in Ethiopian folk medicine” is rephrased to “hence the findings of this study showed Embelia schimperi appears to possess some anthelmintic activity that may support the usage of these plants by local traditional healers to treat helminthic infestations”.
Keywords

Avoid the use of word(s) which have appeared in the title to help improve the visibility of the manuscript after publication

Response

*Embelia schimperi*, which appeared on the title is deleted from the key words and substituted by anthelmintics

Background:

Authors need to improve on the language for better flow. Sentences must be properly synthesized to bring out the convey messages in a logical manner.

Few examples are highlighted below:

Line 73-74: Both sentence can be easily synthesized nicely and combined for better flow.

Background:

Response: The two sentences are combined as “Helminthiases have been affecting human beings for quite long time in the history of mankind and still continue to be the major causes of mortality and morbidity to over a billion people particularly in developing regions of the world like the sub-Saharan Africa (SSA), Asia and the Americas”.

Lines 79-84: A very short paragraph which appears disjointed. The message can easily be incorporated with the one above.

Response: The second paragraph is combined with the first paragraph

Line 91: Insert a comma between ‘medicine’ and ‘scientific’

Response: comma inserted

Line 92: Please replace the word ‘generate’

Response: The word ‘generate’ is deleted and the sentence is restructured.
Methods Line 111: Avoid starting a sentence with ‘number’

**Response:** The sentence is restructured

Line 114: delete ‘gummy’

**Response:** deleted

Line 131: delete ‘very’

**Response:** deleted

Line 148: please convert 2000 rpm to the SI unit (X g)

**Response:** 402 ×g is mentioned instead of 2000 rpm

Results Lines 208-209: remove the capitalization of the phytochemicals

**Response:** This part has been left out

Line 231: insert space between number and units. Check and do same throughout the manuscript

**Response:** space has been inserted between numbers and units throughout the manuscript

Discussion Line 240-242: The sentence add no value, more like a repetition from the introduction, I suggest deletion.

**Response:** The sentence is deleted

Conclusion Perhaps, authors should considered merging as with discussion, the concluding statement is so predictable and shallow.

**Response:** The conclusion is restructured and some points have been added

List of abbreviation Please remove Standard abbreviations (e.g. microgram, microliter, LD50, etc) from the list

**Response:** Those standard abbreviations are removed from the list
Figures: The caption for all the figure are not well explanatory and need to be improved to convey whatever message the authors want to convey.

Response: The captions are restructured to make them more explanatory

Line 369: HPLC should be written in full

Response: HPLC is fully written

Figs 2 and 3: Authors must justify the relevance and value of having these figures in the manuscript. Does it add any extra value to their work?

Response: we reviewed the significance of the table and found out that since the LC$_{50}$ values are indicated in Table one, we decided to leave figure 2 and 3 out.

Table In column 2, instead of repeating ‘Kg’ all the way, it can be captured in the column title “Dose administered (Kg)”

Response: The mg/kg units in each row of column 2 in table 2 and 3 are removed and outlined in the column title
Reviewer: Lyndy McGaw

Major compulsory revisions:

1. The most recent guidelines of the OECD for the in vivo acute toxicity study should have been followed as the method used is rather outdated.

Response: We conducted the acute toxicity study at some years back. At that moment we were using the older version of the OECD guideline.

2. It appears that some available literature concerning the known chemical constituents of the plant species under study was not consulted and a more comprehensive literature search needs to be carried out.

Response: Some important literatures have been consulted to make the discussion more comprehensive.

3. Why was the phytochemical screening performed as it is not discussed in detail and the relevance is lacking?

Response: We found this section not relevant to the study as per the recommendations of reviewers, so we left it out.

4. In the Methods section, where the phytochemical screening tests are described, what are the references for the techniques used?

Response: We found this section not relevant to the study as per the recommendations of reviewers, so we left it out.

5. How many replicates were carried out at each concentration for the in vitro anthelmintic assay?

Response: Each experiment in the in vitro studies was replicated 3 times.
Minor essential revisions:

1. In the methods section of the Abstract, the species of hookworm tested needs to be mentioned.

   Response: the hookworm species is *Necator americanus* and it is corrected accordingly.

2. In the results section of the abstract (final sentence) it should be made clear which assay is being referred to.

   Response: the sentence has been restructured as “The *in vitro* anthelminthic activity study revealed that the LC$_{50}$ value of the crude extract and albendazole were 228.7 and 51.33 µg/mL, respectively” to refer the *in vitro* experiments.

3. Is a dose of 1000 mg/kg of crude extract (or 750 mg/kg of the purified embelin) realistic to achieve in humans? This should be discussed in terms of applicability of the extract in human health.

   Response: a statement has been added addressing this issue in the conclusion part.

4. In line 143, what species of hookworm were tested, and were other species such as roundworms also present? Where was the stool sample obtained (no detail are given in this section)?

   Response: Only hookworm (*N. americanus*) was used in the study. The stool sample was collected from patients in Wolkite health center, 150 km Southwest of Addis Ababa. It is now mentioned in the manuscript.

In line 148, give the speed of centrifugation in “x g” rather than “rpm” and note the type and make of instrument used. With what was the concentration of larvae adjusted – a type of buffer?

Response: rpm is now converted into ×g.

2000 rpm = 402 ×g with rotor radius of 9 cm

5. In line 153, from which company was the albendazole sourced?

   Response: the source is sigma Aldrich and now it is mentioned in the manuscript.

6. In line 156, what type of agar was used?
Response: The agar used was nutrient agar. It is mentioned in the manuscript

7. Please include protocol numbers for the ethical approvals obtained.
Response: Protocol number is now included.

8. In the Results section where the phytochemical analysis is described, what is meant by “chromophores” and “free-quinones” as these are not classes of compounds. The NMR spectra of embelin must have been previously published – insert a reference to which the chemical assignments can be compared.
Response: The phytochemical analysis section is left out
A reference has been cited regarding the NMR spectra of embelin.

9. The Discussion section (line 257) refers to bioassay-guided fractionation being used to isolate embelin but this was not the case according to the Methods section. Please correct this.
Response: since we did not follow bioassay-guided fractionation we deleted and restructured the sentence

10. Also in the Discussion, reference is made to other anthelmintic studies on the extract and embelin but more detail needs to be given and this section could be more clearly explained.
Response: More literatures are consulted to make the discussion more comprehensive

11. In the Conclusion, care should be taken not to state that the results of the in vitro study and an in vivo mouse assay can be extrapolated to efficacy in humans as many other factors play a role, for example dosages could be quite different, and human studies have yet to be carried out.
Response:
The conclusion is restructured and some points have been added testifying the need of further studies prior to recommending it for human use.

12. Grammar needs minor revision by a native English speaker
Response: revision has been made