Author’s response to reviews

Title: Antibacterial activities, proposed mode of action and cytotoxicity of leaf extracts from Triumfetta welwitschii against Pseudomonas aeruginosa

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Answers to reviewers comments

Reviewer 1:

Carlos Henrique Gomes Martins, Ph.D. (Reviewer 1): The manuscript describes the antibacterial activity, the possible mechanism of action and the cytotoxic evaluation of the vegetal species of Triumfetta welwitschii.

1. The article needs a revision in English language and also it needs to be clarified and corrected in some points so it can be published in BCAM.

A revision in English language has been done. Clarifications and corrections in some points have been done.

2. The experiments were made with only crude extracts of the studied vegetal species and the majority of the assays were made with one standard strain (P. aeruginosa) of bacteria, which was taken into account since it presented the best results for the antibacterial activity.

Yes, P. aeruginosa presented the best results thus it was used for subsequent assays.

3. The manuscript does not make clear which the main chemical classes of secondary metabolites are, and what so more the antibacterial action had been already studied in this vegetal species, thus, presenting in this study other assays even though it was made in only one bacterium.

The main chemical classes of secondary metabolites in T. welwitschii have been included. It has been clarified that antibacterial action that has been already studied involved roots of the plant in this vegetal species. This study focused on the leaves of this vegetal species.
4. Other points of fragility in the study

Background

The reading of this item is tiring and does not present the state of the researches made with the T. welwitschii, yet, more than half of this item was used with the intention of justifying the use of certain techniques in the study containing unnecessary information for a specialized reading public.

The state of the researches made with T. welwitschii has been presented. The unnecessary justification of the use of certain techniques in the study has been removed.

5. It is necessary to clarify what are the secondary metabolites found in T. welwitschii what would collaborate with the discussion of the results presented.

The main chemical classes of secondary metabolites in T. welwitschii have been included and collaborated with the discussion of the results presented.

6. Other important question is that the reason of choice of the bacterial species evaluated since the authors inform that the T. welwitschii is traditionally used to treat diarrhoea. Would it not be better to choose bacteria that cause diarrhoea?

The antibacterial action of bacteria (E. coli and B. cereus) that cause food poisoning resulting in diarrhoea had been already studied in this vegetal species thus the shift to common nosocomial pathogens which are a health burden. It has been clarified that roots are used to treat symptoms of diarrhoea and this study focuses on the leaves.

7. Methods

Preparation of extracts

In this item the authors should present a reference to the extract's preparation.

References have been included.

-What was the yield of each extract evaluated?

The yield of each extract has been included.

8. Microbial strains and culture media

- What is the origin of the clinical isolated? Were they isolated from what type of infection and what is the anatomic localization?
The medical microbiologist from Parirenyatwa could only provide information for some of the isolates. The type of infection and anatomic localisation was included for those microbes with available information.

9. Page 6, Line 5: correct Klebsiella pneumoniae

Correction has been made.

10. Determination of antibacterial activities of leaf extracts isolated from Triumfetta welwitschii

The assay needs a literature reference.

Reference has been included.

11. Were all bacteria evaluated with the same culture medium (TBS)? How the results of this technique are interpreted and presented?

Yes all bacteria were cultured separately in TSB. The presentation and interpretation of results for the technique used have been included.

12. To the assays "Determination of the possible mode of action of antibacterial" and "Evaluation of the toxicity of the leaf extracts".

Authors should inform what criteria were used to choose the evaluated bacteria in these tests.

No bacteria were used for both assays. The basis of using the mouse peritoneal cells and sheep erythrocytes is included in the discussion.

13. Page 7, line 24: Confirm the use of the reference 15 in this technique.

Reference alignment has been done.

14. Results

Page 11, lines 15 to 52: Some results are presented in inhibition percentage of the growth and some others in MICs (line 44). The presentation of the results should be standardized.

Presentation of results has been standardised.

15. What represents higher or lower growth inhibition and what reference was used in this comparison?

Use of higher, lower and references used in the comparison has been clarified.
16. Page 12, lines 48 to 58: What was the base used by the authors to describe higher haemolytic effect?

The base used to describe higher haemolytic effect has been included.

17. Discussion

In general, the discussion is based only in studies of others vegetal species, which is in majority references to isolated compounds and there is not technical information of the technique used in this discussion.

The discussion does not make the results interesting.

The discussion has been rewritten to include techniques used and made more interesting.

18. Once more, authors claim that T. welwitschii is a traditional plant used to treat diarrhoea symptoms, but the study is presented with bacteria that provoke other diseases. It is necessary to justify the choice of tested bacteria in the antibacterial activity test.

Choice of tested bacteria has been justified. The antibacterial action of bacteria (E. coli and B. cereus) that cause food poisoning resulting in diarrhoea had been already studied in this vegetal species thus the shift to common nosocomial pathogens which are a health burden. It has been clarified that roots are used to treat symptoms of diarrhoea and this study focuses on the leaves.

19. Authors have lost the opportunity to correlate the results of the standard strains with the clinical isolated. The discussion should also be done considering the structural differences between Gram-negative and Gram-positive that was evaluated.

Correlation of results of the standard strain and clinical isolates has been included.

20. What are the possible secondary metabolites of T. welwitschii that could justify these results?

Secondary metabolites that could justify these results have been included.

21. Conclusion

Concerning the antibacterial activity in the conclusion, it was conducted by the results found to one bacteria (P. aeruginosa), not being permitted the extrapolation of these results.

Extrapolation of results has been removed.

Sunita Dalal, Ph.D (Reviewer 2): Work done is appreciable.
22. Extraction methods could be detailed more.

More detail has been added to extraction methods.

23. Besides references, please include details on methods followed in toxicity studies.

Details on methods followed in toxicity studies have been included.

24. Discussion could be more comprehensive.

Discussion has been rewritten to make it more comprehensive.

Douglas Monteiro (Reviewer 3): The present article evaluated the antimicrobial and cytotoxic effects of extracts from Triumfetta welwitschii. The subject addressed is interesting and relevant from the clinical point of view, however, several aspects must be considered by the authors, as follows.

25. The entire article must be revised for English.

The entire article was revised for English.

26. Introduction. The Introduction section should contain the clinical relevance of Pseudomonas aeruginosa as a pathogen causing infections in humans.

The clinical relevance of Pseudomonas aeruginosa as a pathogen causing infections in humans has been included.

27. Preparation of extracts. A reference should be added to support the method used (The cold maceration method).

Reference has been included.

28. Page 6, lines 19-22. "Inoculum concentration was adjusted to 10^6 c.f.u/ml by diluting the inoculum using tryptic soy broth using McFarland standard". What was the McFarland standard used?

0.5 McFarland standard has been included.

29. Page 7, line 1. "The MTT assay was used to observe the presence...cells". Please add a reference to the method used.

Line has been removed.
30. Page 6. The incubation period to evaluate antibacterial activity should be mentioned.

Incubation period has been included.

31. Why was the antibiofilm effect of the extracts not performed? This analysis would increase the impact of the study.

It is work carried out in a separate study to be published.

32. Page 7, lines 31-33. "The optical density of the cells was adjusted to an OD600 = 1.5 using PBS". The cell concentration in this OD should be mentioned.

The cell concentration in this OD has been included.

33. Results (Page 12, lines 35-42). "The highest amount of nucleic acid leakage was produced when cells were exposed to 200 μg/ml DCM/methanol leaf extract while the ethanol leaf extract caused the least nucleic acid leakage at the same concentration". Is this statement correct? It seems to differ from the results shown in Figure 4.

The statement has been corrected.

34. Page 12, lines 50-53. "At a concentration of 100 μg/ml, the DCM/methanol leaf extract showed the highest haemolytic effect". This sentence should be removed because it is repeated.

The sentence has been removed.