Author’s response to reviews

Title: Antituberculosis activity, phytochemical identification of Costus speciosus (J. Koenig) Sm., Cymbopogon citratus (DC. ex Nees) Stapf., and Tabernaemontana coronaria (L.) Willd. and their effects on the growth kinetics and cellular integrity of Mycobacterium tuberculosis H37Rv

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Editor-in-Chief
Journal of BMC Complementary & Alternative Medicine

Dear Professor/Sir/Madam

Submission of Revised Original Research Article
Anti-tuberculosis activity, phytochemical identification of Costus speciosus (J. Koenig) Sm., Cymbopogon citratus (DC. ex Nees) Stapf., and Tabernaemontana coronaria (L.) Willd. and their effects on the growth kinetics and cellular integrity of Mycobacterium tuberculosis H37Rv

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With regards to the above, we hereby submit our revised manuscript for publication in the Journal of BMC Complementary & Alternative Medicine. All comments by the reviewers have been addressed as listed below. Corrections/insertions/deletions made are highlighted in red in the manuscript.

This manuscript described the investigation of three Malaysian ethnobotanical plant species as potential sources of new potent anti-TB agents. The plant species were: Costus speciosus, Cymbopogon citratus, and Tabernaemontana coronaria. The aims of the research study were to investigate the in vitro anti-TB activity of different solvent partitions of these plants, the phytochemical identification of their most active partitions, and their effects on the growth kinetics and cellular integrity of the tubercle organism.

We declare that the work described has not been published previously, that it is not under consideration for publication elsewhere, that its publication is approved by all authors and the institution where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other languages, including electronically without the written consent of the copyright holder. We also declare that the study is an original work and that the study was performed according to the national and institutional rules considering biodiversity rights. We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Thank you.
LIST OF REVISIONS

COMMENTS BY REVIEWERS, REVISIONS/REBUTTALS, SECTIONS/LINES/PAGES

Kazuhiro Takuma, Ph.D.
(Reviewer 1)

1. Pharmacological mechanisms (overall): How can these compounds exert antituberculosis activity? Which molecules are the targets for these compounds? I strongly recommend you to identify pharmacological mechanisms underlying their antituberculosis activity to make clear the advantage of these compounds compared with isoniazid.

Our study was still at preliminary stage to investigate the partitions of the plant materials and identification of possible compounds present in the active partition. Isolation and purification of the compounds were not carried out yet. Therefore, anti-TB activity of the compounds was not determined. Hence, their pharmacological mechanisms could not be identified. The following explanations were rephrased/inserted in the manuscript.

i. In this study, preliminary phytochemical analysis was carried out to identify the compounds that could be present in the most active partition of each plant. [Results Section: Lines 309-310, Pg 13]

ii. Ideally, the specific bioactive constituents should be isolated and elucidated. At present we are working with our research collaborators to further isolate and elucidate these constituents, which require an arduous lengthy chemical-biological approach. Nevertheless, with the recent availability of a number of modern sophisticated hyphenated separation and spectroscopic techniques, their isolation and elucidation should become much easier. With the isolation of these active constituents, further assays could be carried out to identify the mechanism of their actions on the tubercle cells. [Discussion Section: Lines 487-494, Pg 20]
2. Statistical analysis (Fig. 2): You should carry out statistical analysis for the data of Fig. 2.

Statistical analysis of standard deviation (STDEV) was carried out for the data of Fig. 3 (original Fig.2). At some points it was not clearly observed as the deviations were too small. Fig. 3 was extrapolated from data in Table 3. STDEV data was listed in the table. Presence of statistical analysis was acknowledged by Reviewers 2 and 3. [Table 3 (Pg 37) and Fig. 3]

3. References: There are a great number of papers. Please select the important papers within 50 ones.

The number of references have been reduced from 72 to 58. Changes to the reference number were made throughout the manuscript and were highlighted accordingly. [Introduction Section: Lines 100, 109, 113, Pg 5; Materials & Methods Section: Line 134, Pg 6; Line 177, Pg 8; Lines 201, 206, 215, Pg 9; Line 240, Pg 10; Line 250, Pg 11; Results Section: Line 379, Pg 15; Line 381, Pg 16; Line 411, Pg 17; Discussion Section: Line 453, Pg 18; Lines 458, 460, 470, 472, Pg 19; Lines 483, 499, Pg 20; Lines 505, 507, 510, 515, Pg 21; Lines 538, 543, 550, Pg 22; Lines 560, 568, 569, 574, Pg 23]

Mohamed Abdel-Daim, Ph.D. (Reviewer 2)

1. The manuscript should be revised for linguistic and style error.

The manuscript was revised for linguistic and style error as highlighted. Many sentences were rephrased/inserted/deleted accordingly.

[Abstract Section: Lines 39-42, Pg 2; Introduction Section: Lines 85-86, Pg 4; Lines 93-94, Pg 4; Line 95, Pg 4; Lines 100-105, Pg 5; Lines 110-115, Pg 5; Materials & Methods Section: Lines 201-205, Pg 9; Lines 214-215, Pg 9; Lines 221-223, Pg 9-10; Lines 232-233, Pg 10; Lines 252-253, Pg 11; Lines 263-265, Pg 11; Lines 276-278, Pg 12; Results Section: Lines 309-310, Pg 13; Lines 312-313, Pg 13; Lines 341-343, Pg 14; Discussion Section:

Lines 446-449, Pg 18; Lines 457-458, Pg 19; Lines 461-464, Pg 19; Lines 487-494, Pg 20; Lines 515-518, Pg 21; Lines 538-541, Pg 22; Lines 546-550, Pg 22; Lines 552-554, Pg 22-23; Line 568, Pg 23; Lines 572-577, Pg 23]

2. Abbreviations all over the manuscript should be rechecked. Avoid abbreviations in heading and subheading as well as the beginning of paragraphs.
All abbreviations were rechecked throughout the manuscript. All abbreviated terms were spelt in full in headings, subheadings, tables and figures. One abbreviation at the beginning of a paragraph was amended. [Materials & Methods Section: Line 155, Pg 6; Line 159, Pg 6; Lines 172-174, Pg 7; Line 236, Pg 10; Line 256, Pg 10; Results Section: Lines 283-284, Pg 12; Table 1(title), Pg 12; Fig 1a-c (title), Pg 13; Table 2a-c (title), Pg 14; Fig 2 (title), Pg 14; Line 356, Pg 15; Table 3 (title), Pg 15; Fig 3 (title), Pg 15; Table 4 (title), Pg 15; Line 407, Pg 17; Fig 4a-c (title), Pg 17; Discussion Section: Line 476 (abbr. beginning of paragraph), Pg 19; Table 1, Pg 33; Tables 2a-c, Pg 34-36; Table 3, Pg 37; Table 4, Pg 38]

Parveen Bansal, Ph.D. (Reviewer 3)

1. Update the discussion with recent references

Updated with the addition of 3 recent references. [Discussion Section: Line 470, Pg 19; Line 472, Pg 19; Line 483 Pg 20;References Section: Ref. 38, Pg 30; Ref. 40, Pg 30; Ref. 43, Pg 30]

2. Rewrite Conclusion

i. The n-hexane partition of the plant materials exhibited promising in vitro anti-TB activity against M. tuberculosis H37Rv. Their anti-TB activity was supported by their destructive effects on the integrity of the mycobacterial cellular structure. [Abstract Conclusion: Lines 56-58, Pg 3]

ii. The n-hexane partition of the plant materials exhibited the highest inhibitory activity against M. tuberculosis H37Rv indicating that the active phytochemical constituents could be of lipophilic nature. This indication was supported by the identification of many lipophilic constituents in the partitions using GC-MS analysis. The high killing rate over time shown by the n-hexane partitions of C. speciosus stem-flower and T. coronaria leaf indicates that the active constituents in these plant partitions could serve as sterilising agents against the mycobacterial cells during both lag and log phases of growth. The mycobacteriostatic activity of n-hexane partition of C. citratus stem-rhizome was comparable to isoniazid and its mycobactericidal activity could be time-dependent. Finally, the lethal effects of all the n-hexane plant partitions altered the normal mycobacterial cellular structure and caused cell lysis, thus, prevented proliferation of the cells. [Conclusion Section: Lines 580-590, Pg 24]
3. Figures of GCMS spectra used for identification of n-hexane partition based major compounds have to be included in the manuscript.

Figure 1a - c Gas chromatography-mass spectrometry chemometric profiles of plant n-hexane partitions.

[Results Section: Lines 312-313, Pg 13; Lines 321-322, Fig. 1a-c, Pg 13]

4. Under the heading "Identification of phytochemical compounds in active n-hexane partition", author have mentioned GCMS chemometric profiling. Kindly elaborate the same.

Elaborated accordingly as highlighted in red. [Results Section: Lines 333-354, Pg 14-15]

5. Authors have not mentioned the activity of n-hexane, methanol, chloroform, n-butanol, ethyl acetate. These may have their own anti-bacterial activity. In the manuscript there is no supporting data showing nullification of their own activity.

Assay on the solvents against the test organism was not carried out as precautions were taken in the preparation of the partitions to ensure that all remaining solvent residues had completely evaporated.

i. The pooled n-hexane partition was dried completely in an oven at 40 °C, weighed, and stored at 4 °C prior to usage. [Materials & Methods Section Lines 144-145, Pg 6]

ii. All the plant partition samples were dried thoroughly in an oven at 40 °C to ensure that any remaining solvent residues evaporated completely. This important step was taken to eliminate the intrinsic toxic effect of the solvents on the test organism, which could interfere with the assay results. [Discussion Section: Lines 442-445, Pg 18]