Author's response to reviews

Title: Synergistic anticancer effects of a bioactive subfraction of Strobilanthes crispus and tamoxifen on MCF-7 and MDA-MB-231 human breast cancer cell lines

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Author's response to reviews: see over
Dear Editor,

Thank you for inviting us to re-submit our manuscript entitled “Synergistic anticancer effects of a bioactive subfraction of *Strobilanthes crispus* and tamoxifen on MCF-7 and MDA-MB-231 human breast cancer cell lines” to BMC Complementary and Alternative Medicine.

We thank the reviewers for their kind review and valuable suggestions. We have addressed the concerns raised by the reviewers and we have provided detailed point-by-point responses below. The manuscript has been revised and corrected (with track changes) based on the comments given. We have also included some formatting corrections and these are indicated in the manuscript. We hope that our submission now meets the high standard that the journal requires.

Thank you

Sincerely,

NS Yaacob, PhD
Corresponding Author
Response to Reviewer 1

1. The idea to combine natural herbs with chemotherapies to reduce the side effects of chemotherapy for cancer treatment is very interesting and has good potential.

   We thank the reviewer for the positive comment.

2a. There is not enough information to let readers know what SCS is, as mentioned in the materials and methods section “The SCS was prepared as previously reported [13]”. I am guessing the SCS is SC/D-F9 but it needs to be made clearer.

   We thank the reviewer for pointing this out. Yes SCS is SC/D-F9. The earlier publication (reference 13) compared 15 different sub-fractions of the dichloromethane extract and since only one of those sub-fractions was further tested, it was important to differentiate it (given the laboratory code, SC/D-F9) from the rest. The current manuscript reports further studies on this particular sub-fraction that was prepared in the same way as reported in reference 13. In order to avoid confusion, we have included the statement “(previously coded as SC/D-F9)” in the revised manuscript

2b. The author should provide more information about SCS, such as the active ingredients or major components.

2c. In addition, the author should provide a HPLC fingerprint of SCS.

   We agree with the reviewer that the components of SCS would be of great interest. The process of isolation, characterization and identification of the chemical constituents of SCS is being carried out using various chromatographic techniques, NMR and LCMS which is a long and fairly laborious process. We are therefore not able to report on the constituents at this stage. We have therefore added this statement in the manuscript: “The chemical constituents of SCS are currently being isolated and identified”

2d. The methods of this study should be appropriate and described well enough to allow others to evaluate and/or replicate the work

   The method for the preparation of SCS has already been described in reference 13. Assessments of cytotoxicity, apoptosis, caspase activities, changes in the mitochondrial membrane potential and DNA damage were performed using commercially available kits and recommendations by the manufacturers were strictly followed. Hence, as is customary, the methods were only briefly described.

Response to Reviewer 2

1. Suggest to add a sub-topic as "Chemicals" to list down the chemicals used and its supplier under Materials and Methods (Page 5).

   Thank you for the suggestion. We have included this section in the revised
2. Suggest to add-in the plant authentication and extraction method to prepare sub-fraction.

We agree that we should have added a statement on the plant authentication although we had mentioned this in a previous publication (reference 13) – please see below.

The extraction method was already reported in reference 13 and we had referred to this reference in the Materials and Methods section on page 5.

3. Expand the significant difference levels to include p<0.01 and p<0.001 from the methodology part on page 7.

p<0.05 is considered as the minimum level of significance which automatically includes p values of less than 0.01 and 0.001.

4. Author to reconsider the appropriateness of using Students' t-test or other post-hoc test to analyse the data consisting more than two groups.

Thank you for the comment. We have re-analysed the data using one-way analysis of variance (ANOVA) followed by post hoc Tukey multiple comparison test. The relevant sections have been edited based on this analysis. However, it is noteworthy that the re-analyses using different statistical methods did not alter the outcome of the findings.

5. Author to insert the significant difference level to support the results part.

Thank you for the suggestion. The values have been included in the revised manuscript.

**Response to Additional Revision Request**

Please provide details in your manuscript on the source of the *Strobilanthes crispus* used in your study. We would also ask you to detail whether the plant material was formally identified in any way, and by whom. Please also confirm whether a voucher specimen of the plant has been deposited in a publicly available herbarium, and include this information in your manuscript. A deposition number should also be included, if available.

We agree that we should have added a statement on the plant source and authentication although we had already mentioned this in a previous publication (reference 13). We therefore include the following section under Materials and Methods, in the revised manuscript:
Plant material
The *S. crispus* plants were collected from Tasek Gelugor, Pulau Pinang, Malaysia and authenticated by Mr Baharuddin Sulaiman, a taxonomist at the School of Biological Sciences, Universiti Sains Malaysia. A voucher specimen of the plant (no. 11046), was then prepared and deposited at the herbarium of the School of Biological Sciences [13]