Author's response to reviews

Title: Induction of cell cycle arrest and apoptosis in caspase-3 deficient MCF-7 cells by Dillenia suffruticosa root extract via multiple signaling pathways

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Author’s response to reviews: see over
Dear Editor:

Following your letter regarding the manuscript MS:2003450862114442 entitled Induction of cell cycle arrest and apoptosis in caspase-3 deficient MCF-7 cells by Dillenia suffruticosa root extract via multiple signaling pathways, we are sending the rebuttal letter explaining the changes performed on the manuscript. The changes incorporate the suggestions of the reviewers. We have addressed all the changes recommended by the reviewers and we are confident that the new version of the manuscript is easier to understand and has a more fluent scientific discourse. The revision is addressed as follows.

Reviewer Dr. Yasmin Anum Mohd Yusof

Minor Revisions

1. This is in reference to the multiple signal pathways suggested in the title, and authors need to specify what findings specifically in their previous study to justify the present study besides stating the cytotoxic effects.

   Dear reviewer, for your information, Dillenia suffruticosa root extract has been traditionally and currently used in Malaysia to treat cancerous growth including breast cancer\(^1\). Nevertheless, there is no pharmacological study on the anti-breast cancer properties of the root extract in the literature. In addition, our previous study was only reporting the antioxidant activities of DCM-DS and its cytotoxicity towards several cancer cells\(^2\). Since DCM-DS showed a strong cytotoxicity towards MCF-7 breast cancer cells, therefore the present study was carried out to evaluate the mode of cell death and signalling pathways of DCM-DS in MCF-7 cell line. Please kindly refer to page 5, line 17-25 and page 6, line 1-8.

2. All the pathways described are possible pathways that have been suggested in carcinogenesis; however the authors failed to state which natural compounds activated those pathways, so that it can be compared with their study.

   Dear reviewer, for your information, to the best of our knowledge, the bioactive compounds present in the Dillenia suffruticosa root extract has never been reported in the literature before. Therefore, at this moment, we do not have any information about which natural compounds are responsible to activate the signalling pathways in the present study. Therefore, a study to identify the active compounds that are responsible for the cytotoxicity in MCF-7 cells will be carried out. Nevertheless, based on qualitative analysis of the phytochemicals in our previous study\(^1\), triterpenes appear to be the major compounds in DCM-DS. With that, we have included several triterpenes compounds that activate the signalling pathways in cancer cells as reported by other researchers, which could be comparable to our present study. Please kindly refer to page 22, line 9-16.

3. Seldom in BMC papers are chemicals described as such, please refer to samples of BMC papers whereby many authors state the reagents company and place of manufacturing within the appropriate sub title of methods.

   Dear reviewer, thank you for your comment. The reagents’ company and place of manufacturing have been included within the appropriate sub title of methods.
References


Reviewer Dr. Jong Bin Kim

Minor revision

1. Authors show that MCF-7 cell is caspase-3 deficient through other paper. Authors have to confirm level of caspase-3 of MCF-7 cell through detection of gene level or protein comparing to MCF-10A cells.

Dear reviewer, we have evaluated the caspase-3 level of MCF-7 and MCF10A cells that were used in the present study by using gene expression analysis prior to the conduct of present study. The result showed that there was no detectable caspase-3 in MCF-7 cells at mRNA level. Nevertheless, there was a detectable caspase-3 in MCF10A cells. Previously we did not include this preliminary data in the present manuscript. With that, we have stated the preliminary data in the discussion section as an unpublished data to aware the potential reader of our manuscript. Please kindly refer to page 19, line 13-16.

2. As some reviewer’s comment, DMSO is very toxic compound. Although DMSO non-toxicity reported other paper, authors have to show non-toxicity of DMSO concentration used in this experiment.

Dear reviewer, we have carried out cell proliferation assay (MTT assay) to evaluate the inhibitory effects of DCM-DS towards MCF-7 cells with DMSO at both 0.1 and 0.3% (unpublished data) in our first revision. The growth rate of MCF-7 cells at 0.1 and 0.3% DMSO was not significantly different from the untreated-MCF-7 cells (the control without DMSO and DCM-DS). In addition, the control cells treated with 0.3% of DMSO (Figure 2) grew healthily. Furthermore, we obtained similar IC$_{50}$ of DCM-DS at both percentages of DMSO. With all these evidences, we strongly believe that the DMSO concentration used in the present study was not toxic to the MCF-7 cells. We have included the statement in the text. Please kindly refer to page 8, line 2.

3. In Fig 5, authors should write Manufacture Company of DCFH-DA.

Dear reviewer, the manufacturer of DCFH-DA has been stated in Figure 5.

4. In Fig 6, authors did not experiment for reviewer’s comment about protein level. Based on results of gene level, they show signalling pathway. Protein level is very important to confirm results of gene level. If they show signalling pathway in this manuscript, they should measure protein level.

Dear reviewer, we have performed Western blot analysis on selected proteins to confirm the results of gene expression. Please kindly refer to Fig. 6B.

5. To suggest Fig 7, authors have to confirm protein level.

Dear reviewer, we have performed Western blot analysis on selected proteins to propose the pathways. Please kindly refer to Fig. 6B

6. Reference should correct in line space.

Reference has been corrected in line space.