Author's response to reviews

Title: Cytotoxicity of Selected Cameroonian Medicinal Plants and Nauclea pobeguinii Towards Multi-Factorial Drug-Resistant Cancer Cells

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Author's response to reviews:

Detailed responses to reviewer’s Comments for MS: 2075344625159266

Dear Editor, Dear Tom,

Thanks for sending the reviewer’s recommendations for the above manuscript. We are now resubmitting the revised version taking in account their recommendations to improve it. The revision made are highlighted in recolor color in the manuscript and also listed point-by-point below.

I hope you will found the work suitable for publication now

best regards

(Prof. Dr. Victor Kuete)

Editorial comments

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Please remove your figures from your manuscript document. The figures that you have uploaded into our system are automatically included at the end of your manuscript when the PDF version generates. In addition, we would ask you to please remove the legends from the figures that you have uploaded. The legends should instead be included as a list at the end of your manuscript file.

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Answer: This has been done

Reviewer#1: I. Darren Grice

Reviewer's report:
I believe that all the suggestions made here are important (although minor) for the manuscript to be coherent and sound. The authors could correct/alter these. I do not feel that these should be published with the manuscript – as really they are all of a minor nature, but still very important to the quality of the manuscript. Without these issues addressed the paper would reflect poorly on the work. The work itself appears to be sound and worthy of publication.

Answer: Thanks very much, all the suggested correction have been made as indicated below

Minor Essential Revisions- not for publication

1. Abstract: requires some corrections, inclusions, rewriting of sentences (as indicated in attached scanned pdf document). For example the conclusions paragraph needs to be rewritten to include some specific conclusions on the Cameroonian plants studied (apart from Nauclea pobeguinii).

Answer: Thanks very much, all the corrected requetsed in the PDF were done

3. Introduction: requires some corrections, plus mention of Table 1- that apparently relates to this information. (see attached scanned document for comments).

Answer: This has been done

4. Methods: again some typos and inclusions to address (as indicated in attached document). Of particular note is that a sub-section on the structural characterisation of the isolated compounds should be included here (e.g., MS, NMR). Some of this information has been placed in the Results and discussion section - this should be removed to the methods section.

Answer: This has been done

5. Results and discussion: there are numerous corrections, typos, clarifications required throughout this section (as indicated in attached document). Most of these will help to make the section more readily understood. The authors need to include a comment on the findings for the Cameroonian plants other than Nauclea pobeguinii in their final conclusion (the Cameroonian medicinal plants are featured in the title, but overlooked in the final part of the results and discussion).

Answer: This has been done
References: some attention to the journal format for the listed references is required (as indicated in attached document).

Answer: Corrections have been done

Table 1: a few very minor typos to be addressed.
Table 2: a few very minor typos to be addressed.

Answer: thanks. the minor mistakes were corrected

Figure 1: in structure 3, there are atom labels/bonds that are on top of each other.

Answer: thanks. this has been corrected

The sugars in structures 5 and 6 should be presented in a consistent presentation. In the caption Fig.1. should be I believe Figure 1. In the name of compound 5, the ‘resveratrol b-D-glucopyranoside’, should be ‘resveratrol #D-glucopyranoside’.

Answer: thanks. this has been corrected

Figure 2: Fig.2. should be Figure 2. Caption makes mention of compounds (presumably 1-6 from Nauclea pobeguinii) in the cell assay. But there are no results for these in the figure? Pobeguinii should also be Pobeguinii in the figure caption.

Answer: thanks. these have been corrected

Reviewer#2: Mahmoud El-Readi

Reviewer’s report:

The manuscript entitles “Cytotoxicity of Selected Cameroonian Medicinal Plant and Nauclea pobeguinii Towards Multi-Factorial Drug-Resistant Cancer Cells” is good written and suitable to publications after cover the following concerns:

Major
• More details about the protocol of cytotoxicity assay should be added to the methods section, especially the protocol of suspended cell lines such as leukemia cells.

Answer: This has been done. reference to the methods are also available to avoid detailing a completely commonly used method.
• The authors mentioned that CEM/ADR5000 cells and MDA-MB231/BCRP are resistant cell lines. Are there any evident or experiments (e.g. PCR or WB) were done to confirm this information?

Answer: CEM/ADR5000 is the andriamycin resistant subline of CCRF-CEM whilst MDA-MB231/BCRP are breast cancer resistant protein expressing resistant subline from MDA-MB231 as indicated in the sub-section <cell line>. References are provided. In addition, in this manuscript the IC50 values of doxorubicin, the established cytotoxic drug is 195.12 µM on CEM/ADR5000 clearly showing that these are MDR phenotypes.

• In chemicals section, 2 chemicals vinblastine and geneticin are mentioned, while I did not found their roles through the manuscript. Please write their application in the methods sections, otherwise the authors can delete it.

Answer: There was no vinblastine in this study. this has been deleted. Details on cell culture were provided to show the use of geniticin

• The authors used Adriamycin (specific P-gp substrate) as positive control to compare the cytotoxic effect of extracts and isolated compounds. This is ideal with CEM/ADR5000 while in other cells such as MDA-MB231/BCRP it is better if authors use specific BCRP substrate as positive control other than doxorubicin.

Answer: Thanks, this can be taken in account in our future study. However, MDA-MB231/BCRP was more resistant to doxorubicin (adriamycin) than its sensitive subline MDA-MB231, indicating the its use is also appropriate in this cell lines as well as in others as reported in many studies.

• The IC50 values of all tested samples against HepG-2 and normal hepatocytes as compare to other cell lines cause more conflict for me. Previously, I investigated all these cell lines before that and always I found that the IC50 values of our tested staff against resistant cell lines that highly expressed ABC transporters were higher than their IC50 values against HepG-2 !!!!!. Are there any explanations for that?

Answer: Thanks, as we did not performed specific work on this aspect, we do not have any explanation. Therefore, any explanation from our results now will be a speculation.

• In photochemistry part, I recommend to present all spectra data of isolated compounds in table to easily check it.

Answer: All compounds are known. We therefore provide a supporting information with the NMR data.
• The discussion of the cytotoxicity results of isolated compounds is not enough. The authors can discuss in more details the difference in effect of resveratrol and other compounds and between resveratrol and its glycoside according to the SAR.

Answer: Unfortunately, a part of resveratrol and its glycoside, other compounds are chemically unrelated and are not active, except 6. This does not allow SAR study. However, we added some more specifications on the activity of resveratrol and its glycoside in the discussion section.