Author's response to reviews

Title: Studies of the in vitro anticancer, antimicrobial and antioxidant potentials of selected Yemeni medicinal plants from the island Soqotra

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Author's response to reviews: see over
Dear Dr. Puebla

First of all, I would like to thank you and the respected reviewers very much for their valuable comments. I have revised the manuscript and integrated many comments which will put the manuscript in a better shape.

Please note that all revised phrases are highlighted in yellow color.

**Replies to the comments by Dr. Shang-Tzen Chang:**

First of all I would like to emphasize that I respect the decision although I completely don’t agree with. For that I have several reasons. Our article could be with limited or of no interest for researchers far away from our field (natural medicine and natural products). It is true that we published a series of papers with similar topics but not from 2001. I stated in my introduction that the island Soqotra is a unique place that possesses around 300 endemic uninvestigated plant species. Our research group is the first group (may be the sole one) which started with chemical and pharmacological investigation on plants of this island since 2005. We previously reported the antimicrobial, anticancer and antiviral activities of 25 plants in 3 separated papers. This time we would like to publish the pharmacological and phytochemical investigation of further 26 *uninvestigated* species in one paper. I strongly believe that the respected reviewer didn’t pay attention to the importance of this island as a source of huge number of endemic plants. Additionally, he didn’t consider that there are still hundreds of plant species on this island which need to be studied and evaluated pharmacologically and phytochemically. Does the respected reviewer think that we have to stop such screening researches? I carefully feel sure that such research works throwing light upon medicinal plants are very promising in the potential discovery of new natural bioactive compounds. If you don’t agree with that, tell me please how taxol, vincristine, vinblastine, podophylotoxin,…. and other hundreds of natural compounds were discovered.

**Replies to the comments of Dr. Vanessa Sttenkamp**

Firstly, I would like to thank Dr. Steenkamp for her really very valuable comments and precious queries.

I generally noticed that the respected reviewer found that many parts of the paper are too long and must be omitted. So I would like to emphasize that I submitted the paper as a *research article* and not as a short communication or short report. Thus the paper should give more
details and more descriptions than in a short report. Moreover the paper couldn't be published as a short report, since it described several (not one) pharmacological activities and phytochemistry of 26 uninvestigated (endemic plants) from a unique place in the world (Soqotra island).

Abstract:
1- It would like to mention that I just fellow the instructions for authors of the BMC-series journals, which required that the abstract should not exceed 350 words and must be structured into separate sections: **Background**, the context and purpose of the study; **Methods**, how the study was performed and statistical tests used; **Results**, the main findings; **Conclusions**, brief summary and potential implications.

2- The abstract didn't exceed 350 words and i tried to fellow the mentioned divisions in the abstract. Nevertheless, many sentences are deleted in the revision.

3- Large number of researchers concentrates only on the abstract and would like to have the most important information in this part of the paper. I think that plant names are one of the most essential information for the readers, so i strongly believe that it will be a mistake if the abstract doesn't show these plant's names.

4- The names of the cancer cell lines will be added in the revision.

Methods:
5- Plant materials are referred to Table 1 in the revision.
6- The terms "Gram-positive and Gram-negative" will be written in the text.
7- Details of test methods are required in many journals. I had the experience that once i summarized the extraction method and test methods in one of my papers, the reviewers demanded details, even they wanted to know how many ml were used for the extraction. What I wrote was a brief description for the methods. If you read the instructions for authors of BMC-Journals, you will find that they demand a clear description for the methods used.

8- Microbial concentration with which agar was inoculated will be added in the revision.

9- Broth micro-dilution assay was repeated twice just to confirm the results obtained in the first trial. All experiments were done at the Pharmacy faculty-Greifswald-University (Germany). I left Germany 4 months ago, so it will be really impossible to test or to repeat the test again. The MIC values were only determined for Gram-positive, because the Gram-negative bacteria showed in all cases, negative results at 4 mg/disc (with the exception of 2 extracts). So we thought it is not necessary to test against Gram-negative bacteria using Broth micro-dilution assay. Moreover, please don’t also forget that the
highest concentration used in this test was 1mg. I strongly believe that we never had any activity with that concentration against Gram-negative bacteria. Consequently, it is not necessary to test against Gram-negative bacteria, especially that we were looking for interesting results.

10- Using etoposide as a positive control will be added in the text.

11- Actually, we tested only the methanolic extracts against cancer cell lines. From our experience, normally the water extracts didn't show any in vitro anticancer activity, at least at the concentrations used in the screen ($\leq 50\mu g/ml$). We selected some water extracts and tested them at $100\mu g/ml$ without any positive results, so we decided to save effort and time; particularly we are looking for interesting extracts which exhibit high activity at low concentration (criteria of the American National Cancer Institute).

12- Ascorbic acid as a control will be added in the paragraph "Determination of antioxidant activity".

Results and discussion:

13- What was written in the part of "Results", is actually necessary since it is a research article. In this part i gave emphasize to the most important data in all 4 tables. Thus, few and important examples were just taken from the tables and represented in this part. Nevertheless, many sentences are deleted in the revision.

14- I think the first Paragraph in the discussion is important as connecting or entering part to the discussion. It is also modified in the revision.

15- Words like remarkable, extraordinary must be omitted. Actually i read such words in many papers describing pharmacological effects. One of our research group is Prof. Bednaski, who is an American citizen and check the manuscript, didn't notice that these words are far away from scientific reports. Nevertheless, i will omit these words in the revision.

16- Strong antioxidant effect is a high effect (more than 80%) comparable with the effect of ascorbic acid obtained at low concentration e.g. 50 or $100\mu g/ml$. That was mentioned in the results as well as discussion and will be more emphasized in the revision.

17- The difference in our results and results from literature data depends mainly on several factors. One of them is that in many cases different plant species of the same genus were investigated. So a difference in the quantity and quality of the active constituents responsible for the effects should be there. Another important point is the ecological factors effecting the growth of the investigated plants and so the active constituents synthesized by the plant.
18- I don't agree with the suggestion of the respected reviewer to delete Table 2. I believe that will break the sequence of the study and decrease the efforts and value of the work done in this research. Additionally, both results (inhibition zones and MICs) are in most cases connected in many papers and they reflect a consistent antimicrobial effect.

At the end, I would like to thank Dr. Steenkamp for these very valuable comments, which will be taken in this paper and in the future in further scientific works. Moreover, i would really appreciate if Dr. Steenkamp could understand my insistence, some few points not to change for the reasons mentioned above.

**Replies to the comments of Dr. Tsutomu Hatano**

I would like to thank Dr. Hatano for his valuable thoughts and queries.

1- Description to the TLC.

I mentioned in the part of methods that the screening of chemical constituents of the extracts was carried out with by using chemical methods and thin-layer chromatography (TLC) according to the reference (Wagner H, Bladt S:. Plants Drug Analysis: A Thin Layer Chromatography Atlas). It is a book dealing with how active constituents could be identified using TLC. The information taken are distributed in about 60 pages, so it was really difficult to summarize them in few sentences. The extracts were sprayed after development of the TLCs with specific chemical reagents which react with some groups of active constituents giving certain colors. This information is common for us pharmacognosists and phytochemists and i thought it was not necessary to be described. Many readers will find that as superfluous information. In spite of this, few lines were added in the revision.

2- Cytoxic will be corrected to cytotoxic in the revision.

**Replies to the comments of Dr. Claudia Pessoa**

Thanks a lot for the comments.

Again, I would like to thank all respected reviewers for the thoughtful comments and suggestions and hope that my revisions are acceptable for publication in the BMC Complementary and Alternative Medicine.

Sincerely Yours,
Ramzi Mothana