Author's response to reviews

Title: Antibacterial activity of some selected medicinal plants of Pakistan

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Version: 7 Date: 14 June 2011

Author's response to reviews: see over
To,

Chief Editor,

BMC Complementary and Alternative Medicine

Dear Editor,

I am submitting with this letter a revised version of our manuscript “Antibacterial activity of some selected medicinal plants of Pakistan” (MS: 3986070515205425) by Yamin Bibi, Sobia Nisa, Fayyaz M Chaudhary and Zia Muhammad, to be considered for publication in BMC Complementary and Alternative Medicine.

We are grateful to the Reviewers for all their suggestions, which we took into account when preparing the revised manuscript. Below we describe in detail our response to the comments. Fragments of the manuscripts that were changed or added are highlighted in grey.

We hope our revised manuscript meets the requirements for publication in the BMC Complementary and Alternative Medicine. If you require any further information, please do not hesitate to contact us directly.

With sincere regards,

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Response to Reviewers’ Comments

Reviewer 1: Branislav Rankovic

We are very grateful to the Reviewer for the comments, which very much improved our manuscript.

Comment: Section Background is too broad, and in my opinion, it gives a lot, unnecessary details, thus losing the information content. I propose to re-write clearer and less with the most important elements that are directly related to the subject of research.

Response: As directed by reviewer, traditional use and properties have been removed from the text and presented in tabulated form in order to avoid repetition. Further, Background has been improved. Instead of a detailed description of each plant, now we have included the required detail with references in table 1. In the text we have added the following text:

In Pakistan, a vast diversity of bioactive plants grown naturally. In the present study, we have investigated the bioactivity of following six naturally growing plants: Aesculus indica Linn., Arisaema flavum, Debregeasia salicifolia, Pistacia integerrima Stew. ex Brand, Toona ciliata, Carissa opaca. Their distribution, traditional use and properties [10-30] are described in table 1.

Comment: Abstracts section, subsection Methods: In the third line reads ".. thee Gram positive and two Gram negative ATCC clinical isolates. I think it is better to stand for ".. three Gram positive and two Gram negative ATCC bacterial species.

Response: As suggested by the reviewer, correction has been made in the revised manuscript.

Comment: Subsection Antibacterial Activity Not specified percentage dimethyl sulfoxide concentration (DMSO) used as a solvent for dissolving the extract. Unknown is the concentration of the solvent used for dissolving Cefalotaxime which was used as positive control. Also it is not clear what the authors mean by the title clean DMSO that was used as a negative
control, possibly 100%? Concentration of DMSO is very important since higher concentrations have toxic to microorganisms.

**Response:** As highlighted by the reviewee, percentage of DMSO % has been mentioned (see Subsection Antibacterial Activity) in revised version.

**Comment:** Discussion should be a little more extensive, will be spread analyze their results in comparison with recent results of other researchers.

**Response:** As advised by the reviewer, discussion has been improved. Some references [33, 36, 38-41] have been cited with corresponding detail.

**Comment:** References section, there is a misprint in the first author in the other references where improperly writes 2Nathan C., and should be properly Nathan C.

**Response:** As indicated by the reviewer, reference has been corrected accordingly see Ref. [2].

**Comment:** Table 2 there is a misprint in the second row where the plant species incorrectly stated P.pPickettii, and should be properly stated P. pickettii.

**Response:** As pointed by reviewer, typographic mistake has been corrected.

**Reviewer 2:** Ramon Enrique Robles Zepeda

We are very grateful to the Reviewer for the comments, which very much improved our manuscript.

**Comment:** 1) Delete the introduction information on the characteristics of the plants used, because they are already included in table 1.

**Response:** As directed by reviewer, information regarding characteristics of plants have been removed from the text and presented in tabulated form in order to avoid repetition. Further,
Background has been improved. Instead of a detailed description of each plant, now we have included the required detail with references in table 1. In the text we have added the following text:

In Pakistan, a vast diversity of bioactive plants grown naturally. In the present study, we have investigated the bioactivity of following six naturally growing plants: Aesculus indica Linn., Arisaema flavum, Debregeasia salicifolia, Pistacia integerrima Stew. ex Brand, Toona ciliate, Carissa opaca. Their distribution, traditional use and properties [10-30] are described in table 1

Comment: 2) In the Introduction it is mentioned that “The bacterial organisms including Gram positive and Gram negative like Bacillus, Staphylococcus and Micrococcus, Salmonella and Pseudomonas species are the main source to cause severe infections in humans”  
The genus Micrococcus not causes human infections. In the genus Bacillus there is a species that can cause food intoxication (cereus), other highly pathogenic to humans (anthracis, including used for biological warfare), the rest of the species does not cause disease. The genus Bacillus has low importance as an infectious agent (except in the case of biological attack).

Response: As pointed by reviewer, Micrococcus has been deleted from Background section.

Comment: 3) In Table 2, there is an error in the name of Pseudomonas pickettii. In the same table, the title of the table could include that was measured "diameter ".

Response: The reference has been corrected accordingly see Ref. [2]

Comment: 4) Salmonella setuball is not the correct name; you must remove a "l" at the end. That error is found throughout the manuscript. Actually the correct name should be "Salmonella enterica subsp. enterica serotype Setubal.

Response: As mentioned by the reviewer, Salmonella Setubal has been corrected throughout the text in the revised version of the manuscript.

Comment: 5) The last two figures are very confusing; I suggest should make tables and report only the% inhibition above 50.
Response: As highlighted by the reviewers, in order to avoid confusion to the readers Figure 3 has been removed from revised manuscript.

Now we have realized that Figure 2 is enough to explain and compare the results clearly. However, due to large number of sample looks crowded but we did not find any alternative to express and compare the results in a more sophisticated way. In order to understand statistical analysis, it is necessary to include activity of each extract.

Comment: 6) In general, figures should be modified a way important to be clearer and reflect the results without confusion.

Response: See respond to comment 5

Comment: 7) The methodology is well planned but perhaps too detailed. For example, I think no need to mention final pH of the culture medium, or how prepared or how much volume of culture medium powder was weighed, how prepared the turbidity standard, etc.

Response: We agree with the reviewer at this point.

But it is also necessary for the reproducibility of the results due the following reasons:

a) Some time distilled water is of low pH that effect on growth of microorganism.

b) Normally in such assays turbidity is not calculated, that effects on results. Turbidity can be measure by Mcfarland or may be by O.D. so procedure is given.

c) Regarding weight of culture medium; it varies company to company.

d) It is our experience that for 100 µl test sample, 75-80 ml media should be poured in Petri plate. Varying volume of culture media may affect the results. If culture media is less, the sample may spread out of well, if culture media is more, the test sample will not give proper results.
Reviewer 3: Moschos Polissiou

We are very grateful to the Reviewer for the comments, which very much improved our manuscript.

Comment: 1. *It would be helpful to give the chemical composition or the main components of the crude extracts according to the bibliography.*

Response: 1. The phytochemistry of these plants is discussed in discussion part of the manuscript see Refs. [33, 36, 15, 38].

Comment: 2. *Which was the quantity of methanol used for the extraction of the plant metabolites?*

Response: 2. As pointed by the reviewer, quantity of methanol for soxhelt extraction has been mention in revised manuscript. While for cold maceration, quantity of methanol varies for each plant depending upon quantity of plant material. For extraction the plant material was dipped/soaked in methanol.

Comment: 3. *Change ml to mL.*

Response: 3. As advised by reviewer, the ml has been changed to mL throughout the text.

Comment: 4. *The authors make a comment concerning the quantity of the active constituents at the last paragraph of Results and Discussion section. This comment is rather arbitrary.*

Response: 4. As directed by reviewer, the last paragraph has been modified with references [see Refs. 39-41] to support the results.

Comment: 5. *At the conclusion, they said that the methanol fraction of Pistacia integerrima proved active against the tested microbes. This is not supported by Table 2.*

Response: 5. This was a typographic mistake; that has been removed as pointed by reviewer corrected