Author's response to reviews

Title: In vitro antimycobacterial activity of nine medicinal plants used by ethnic groups in Sonora, Mexico

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Version: 2 Date: 15 May 2013

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TITLE:

In vitro antimycobacterial activity of nine medicinal plants used by ethnic groups in Sonora, México

AUTHORS:

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Tom Rowles  
Executive Editor  
BMC Complementary and Alternative Medicine

Dear Dr. Tom Rowles,

I received the reviewers’ comments regarding the manuscript that we sent for publication in BMC Complementary and Alternative Medicine (Manuscript, MS: 1284775545910449, entitled “In vitro antimycobacterial activity of nine medicinal plants used by ethnic groups in Sonora, Mexico”). We have responded to all comments raised by the reviewers, and we hope that our improved revised manuscript is now suitable for publication in your prestigious journal. The modified parts of the paper are highlighted in bold-face type and are aimed at clarifying issues raised by the reviewers.

I look forward to your reply and final decision.

Sincerely yours,

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**Author’s response to reviews**

**Reviewer: Virgilio Bocanegra-Garcia**

**Comments to the Author:**

**Background:**

**Comment 1:** About “Antibiotic misuse”. In the case of antibiotic treatment in TB infected patients, the main problem is not with misuse, but with patient treatment adherence, since when a patient stops taking the antibiotics, because of side effects or for other reasons, resistance arise.

**Answer 1:** We agree with the reviewer’s comment, that the lack of adherence to the tuberculosis treatment promotes antibiotic resistance. Modifications were done in the manuscript to express this affirmation.

**Methods:**

**Plant Collection**

**Comment 1:** All Plants were collected all across the Sonora State, or just in a defined region such as the north or the south? This should be clarified, since the authors state that Sonora is the second largest state in Mexico, and environmental differences (temperature, humidity, soil composition, etc) may be present along the state

**Answer 1:** This is correct, different authors have stated that there could exist an effect over the plant secondary metabolites content related to its place of growth; however, this would only be applied when a comparison among species collected in different places were done; in different species it is not possible to evaluate this effect.

**Comment 2:** Please state if plant were collected from wild environments in the field, since the environmental conditions may have important effects in the metabolites production

**Answer 2:** All plants were collected in wild environments located in the surrounding area of the city of Hermosillo, Sonora. *Phoradendron californicum* was collected in a zone located at 80 km to the South of Hermosillo; both collecting areas belong to the Sonoran desert ecosystem. The three species that presented antitubercular activity (*G. coulteri*, *A. confertiflora*, and *A. ambrosioides*) were collected in this kind of ecosystem.
Comment 3: Please state the final concentration of DMSO in the in vitro assays, since DMSO can have inhibitory effects by itself

Answer 3: Final concentration of DMSO in the assay was of $\leq 5\%$, which does not produce mycobacterial toxicity, as endorsed by our control result.

Results and Discussion

Comment 1: Why not to use the aqueous extract? Since the infusion is the traditional extract methods used, it would make sense, that the aqueous extract should be tested

Answer 1: The extraction method was chosen by taking in consideration that methanol extracts may contain a wide range of chemical compounds with biological activity, such as terpenoids, phenols, flavonoids, saponines, steroids, and others (Tiwari, 2013; Maddan, 2011; Khatuntseva, 2012; Viswanathan, 2012). Also, there have been some studies reporting that the methanol extracts are more active than aqueous extracts in their antibacterial activity (Parekh, 2005).

Comment 2: Author should discuss why the traditionally used plant G. coulteri resulted less effective than A. confertiflora that is not traditionally indicated as anti-TB plant. Also why A. confertiflora is more active than A. ambrosioides, since they both are from the same genus.

Answer 2. Ambrosia confertiflora is used by Sonoran ethnic groups to treat symptoms closely related to tuberculosis, as fever and lack of appetite (Table 1). The family to which it belongs (Asteraceae) contains a high concentration of sesquiterpene lactones (SQL) characterized by their wide variety of biological activities, including antimycobacterial effects (Cantrell, 2001; Seaman, 1982). Moreover, although A. ambrosioides is not used as antitubercular agent by Sonoran ethnic groups, it is used for placenta expulsion (Table 1). Given that the placenta is rich in proliferating cells (Furukawa, 2011) and A. ambrosioides acts on them, this fact may also explain its antiproliferative effect on mycobacterial cultures. The difference of the antimycobacterial activity among A. confertiflora, A. ambrosioides, and G. coulteri could be attributed to the difference in the relative concentrations of the active principle in these plants. However, further investigations are required to demonstrate this statement.
Comment 3: the discrepancy with the Molina-Salinas report could be due to the different polarity of solvent used, so the results are not comparable. Author should state that, and not just do a plain comparison of the results.

Answer 3: To this respect, we have now included the following paragraph in the manuscript. The differences between the results obtained by Molina-Salinas and ours in respect to the S. molle activity on M. tuberculosis can be attributed to the fact that a hexane extract contains mainly non-polar compounds, in contrast to the methanolic extract evaluated by us, where higher polarity compounds are present.

Comment 4: If SQLs are already been reported as possible active anti-tb compounds, why the authors did not apply an analytical test for the measurement of those compounds? Or at least the use of a qualitative test to demonstrate that SQLs are present in their extracts?

Answer 4: Based on studies that point out that both A. confertiflora and A. ambrosioides are rich in SQLs (Cantrell, 2001; Seaman, 1982), we chose to perform the Baljet reaction, which resulted positive for all of our different extracts of A. confertiflora (Table 3) and the methanolic extract from A. ambrosioides. Although this reaction is also positive for cardiac glycosides and others containing α, β-unsaturated lactones (Silva, 1998).

The Baljet reaction methodology has been added to the ‘Methods’ section.

Comment 5: Authors should discuss why despite several SQL have been reported in A. confertiflora, with a low MIC that the one they find in their study, the MIC of A. confertiflora extracts even in less polar solvent is still higher that previous reports?

Answer 5: The MIC values obtained in our study are higher in comparison with the ones previously reported for the SQLs isolated from A. confertiflora (reynosin and santamarine) because the last are pure compounds (Cantrell, 2001), whereas ours were crude extracts.

Comment 6: Since G. coulteri and S. molle has been the ones referred as traditionally used against Tb disease, why authors did not test less polar extracts in that plants to, so possible anti-tb activity could be detected? As it was seen in A. confertiflora, those extracts may have been more active than the methanolic extracts, even according with the Molina-Salinas report.
Answer 6: Although, both *G. coulteri* and *S. molle* have been referred by the Sonoran traditional medicine as antitubercular agents, these plants are used as infusion, thereby the methanolic extract was used, as we previously stated, it allows to extract a wide variety of compounds of diverse chemical nature. In addition, we can comment that references to the use of medicinal plants by ethnic groups are mainly a relevant guide in the research of natural products; however, in many cases it is possible to validate the biological activity referred, whereas in many others it is not possible to confirm scientifically their medicinal property. Moreover, extracts with less polar solvents were generated for *A. confertiflora* because there are reports (Yoshioka, 1970) that refer the presence of SQLs in this species. The antimycobacterial activity has been attributed to this kind of molecules. Further studies are being carried out over *A. confertiflora* to isolate the active compounds.

Conclusions

**Comment 1:** Authors state that the fact that *G. coulteri* was active against in vitro supports the empiric knowledge of its use in traditional anti-tb therapy, but in their results *G. coulteri* was not the most active plant against Tb in vitro since both the Ambrosia genus plants were more active, so, empiric knowledge would be inaccurate and the plants traditional used are not the best available choices. More about this fact should be addressed in the discussion section.

**Answer 1:** This has been included in the manuscript.

**Comment 2:** Authors results indicate that despite infusion is the traditional used method, it is not the best method for extract of the active compounds in those plants, since the extracts with less polarity were more effective, and authors do not indicated that neither in discussion nor in the conclusions.

**Answer 2:** This has been included in the manuscript.

**Comment 3:** Authors should state a brief and possible explanation of why the less polar extracts resulted more active, beyond the fact that SQL are extracted in that fractions, since already reported MICs are lower than the ones authors find in their study, so additional factors may be interfering.

**Answer 3:**

It has been reported that molecules with high polarity present a reduced transport through the outer lipid layer of the mycobacteria, and, in consequence, present lower antimycobacterial activity; whereas, less polar molecules present a higher permeability (Korycka, 2005; Luna, 2007). These reports are in agreement with our results, where the less polar solvent crude extracts showed better activities than the ones presented by the methanolic extract.
Reviewer: Bina S. Siddiqui

Comment to the Author

Comment 1: Authors’ institution name should be in English.
Answer 1: We have changed the institution name to English.

Comment 2: Only eight plants are listed whereas the study has been undertaken on nine plats. Please correct.
Answer 2: We have added the missing plant to table 1.

We considered all of the comments raised by reviewer Bina S. Siddiqui in the revised manuscript. The changes are highlighted in bold text.
Reviewer: Tulin Askin

Comments to the Author

Comment 1: Minor essential revisions, author(s) should check the correct writing of some word such as characterised, authentified, analysed and colaborators.

Answer 1: This suggestion has been considered and the words were changed to ‘characterized’, ‘authenticated’, ‘analyzed’ and ‘collaborators’. Also, a native-English-speaker copy-editing has been done to the whole manuscript.

Comment 2: As a discretionary revision, author might be chosen to give much more ethnobotanical usage of these plants for various diseases.

Answer 2: A deeper discussion over the ethnobotanical usage of the plants has been carried out, particularly in those that shown antimycobacterial activity and the ones that are reported as antitubercular.

We considered all of the comments raised by reviewer Tulin Askin in the revised manuscript. The changes are highlighted in bold text.