Author's response to reviews

Title: Antimicrobial and antiproliferative activities of stingless bee Melipona scutellaris geopropolis

Authors:

Marcos G da Cunha (mgcunhafarma@yahoo.com.br)
Marcelo Franchin (marcelo.franchin@yahoo.com.br)
Lívia CC Galvão (liviagalyao@ymail.com)
Ana LTG Ruiz (analucia@cpgba.unicamp.br)
João E Carvalho (carvalho@cpgba.unicamp.br)
Masaharu Ikekaki (masaharu.ikekaki@gmail.com)
Severino M de Alencar (smalencar@usp.br)
Hyun Koo (hyun_koo@urmc.rochester.edu)
Pedro L Rosalen (m099721@dac.unicamp.br)

Version: 2 Date: 14 November 2012

Author's response to reviews: see over
Dr. Tom Rowles
Executive Editor
BMC Complementary and Alternative Medicine

Ref. MS: 1029151319796237 – Answers to reviewers

Dear Dr. Rowles,

We are re-sending our manuscript entitled “Antimicrobial and antiproliferative activities of stingless bee *Melipona scutellaris* geopropolis” (MS: 1029151319796237) after being thoroughly revised and we hope that now we can meet the high standards of this journal. Please see the answers to the reviewers and comments below. All the changes suggested by the reviewers are highlighted (IN YELLOW) in the manuscript.

We would like to thank you and the reviewers for the comments and the opportunity to revise our paper.

Sincerely yours,

Pedro Luiz Rosalén
Corresponding author
Answers & Comments

Reviewer #1 (Shruti Shukla):

“Comments: I have read the manuscript carefully. The manuscript has presented considerable amount of work. However, there are so many comments which author should rectify prior to accept the manuscript for publication. Also authors reported antimicrobial activity using MIC/MBC determination. In my opinion to improve the quality of the manuscript, authors should design some more experiments to visualize the effect of the antimicrobials using cell viability assay, and SEM analysis to visualize the effect on cell wall integrity."

We are glad about the comments and scientific perspicacity of the reviewer to improve the quality of our manuscript regarding the antimicrobial focus. Indeed we have used these methodologies in another recent paper (Evid Based Complement Alternat Med 2012, DOI: 10.1155/2012/751435). However, considering the unique nature of the product, the present study reflects the preliminary study about a singular endangered stingless Brazilian bee species, whose environmental sustainability is fundamental. Thus this paper has social and environmental importance, since it can help call the attention to the significance of this bee family so that it can be protected by the community, and also strengthen the use of geopropolis by alternative medicine practitioners. Bearing this in mind, we show two biological properties that corroborate its normal use in folk medicine in the state of Bahia, in the Northeast Region of Brazil, and that can add value to this natural product. In this context, the study was designed to answer a question about stingless bee geopropolis: Does it have potential antimicrobial and antiproliferative activities that can be correlated to its use by folk medicine practitioners? Our paper clearly points out that geopropolis presents these two potential activities, showing the significant value of this natural product. The next step in our research should be the investigation about the mechanisms involved in both the antimicrobial and antiproliferative properties of geopropolis, which was not the goal of the present work.
“1. Whether the abbreviations HF, CF and AcF are suitable or not. Please check.”

We usually use these abbreviations in our papers (J Ethnopharmacol 2012; 143(2):709-15), but because of this comment, we searched the literature and found the same abbreviations for hexane fraction – HF (Eur J Nutr 2012, DOI: 10.1007/s00394-012-0345-1; Nat Prod Res 2012, DOI: 10.1080/14786419.2012.698407, and others) and chloroform fraction – CF (Asian Pac J Trop Med 2011, 4(10):786–789; Inflammation 2011, 34(6):630–638, and others). However, as we did not find the abbreviation AcF, we agreed with the reviewer and made the necessary changes in the manuscript, now adopting ethyl acetate fraction – EAF, according to the literature (Nat Prod Res 2012, DOI: 10.1080/14786419.2012.698407; J Ethnopharmacol 2012, 143(1):179–184).

“2. Whether the column temperature 350°C is proper or nt. Kindly justify.”

We apologize for the typo. In fact, the correct column temperature is 35ºC. We made the modification in the manuscript. Please see the “Reverse-phase high-performance liquid chromatography (RP-HPLC)” section in the manuscript.

“3. Why Figure captions are included between the text.”

We apologize for our mistake. The figure captions are in the proper place now, at the end of the document. Please see the “Figure legends” section in the manuscript.

“4. Author also specifies the RI value of identified compounds.”

The Kovats retention index (RI) values of the compounds identified in our study were measured based on a mixture of alkanes (C7-C30) injected under the same conditions of the samples. Please see the second column of “Table 3” in the manuscript.

“5. Give the specification of Instrument used for measuring OD.”
The experiment to check the inhibition of *Streptococcus mutans* biofilm adherence was performed using “a Biochrom Asys UVM 340 Scanning Microplate Reader (Asys HiTech GmbH, Cambridge, United Kingdom) and the ScanPlus 2.0.1 software”. The information was added to the “Inhibition of *Streptococcus mutans* biofilm adherence” section in the manuscript.

“6. *Topological errors and grammatical errors are also there.*”

The manuscript was edited by an experienced professional in order to reach the outstanding standards of this journal. Please see the revised manuscript. These changes were not highlighted in the manuscript.

“7. *What is BHI broth. Kindly explain?*”

The term “BHI broth” refers to Brain Heart Infusion. Now it is properly stated in the “Bacterial strains and susceptibility testing” section, and to avoid misunderstandings, we decided to use the abbreviation “BHI” to describe the broth and “BHI agar” to describe the brain heart infusion agar.

“8. *Kindly provide enough information on HPLC chromatograms.*”

Thank you for the opportunity to add more information to the HPLC chromatograms. Please see the “Figure legends” section at the end of the manuscript.

“9. *References are not set properly.*”

The references were carefully checked and now they are properly set. Please see the “References” section in the manuscript.
Reviewer #2 (Vivek K. Bajpai):

“1. In abstract body, authors have repeated the text, kindly check carefully and revised accordingly.”

We apologize for our mistake. We carefully checked the abstract body and revised it properly. Also, the English language professional double-checked the abstract to meet the high standards of this journal. Please see the “Abstract” section.

“2. In abstract body, the text which authors mentioned in conclusion section, it belongs to results section, hence, author should revise the conclusion section in abstract body.”

We agree with the suggestion of the reviewer and changed the abstract body. Please see the “Abstract” section.

“3. The formatting of the manuscript is very poor. Hence, the whole manuscript should be revised properly.”

The manuscript was edited by an experienced professional in order to reach the high standards of this journal. Please see the revised manuscript. These changes were not highlighted in the manuscript.

“4. There are so many grammatical errors in the manuscript in the present form and the language is very poor. Hence, the manuscript should be checked by a native English speaker who is expert in writing scientific English language.”

As we mentioned in the last response, the manuscript was edited by an experienced professional. Please see the revised manuscript. These changes were not highlighted in the manuscript.
“5. Why authors have provided figure legends in the middle of the manuscript text, they should be placed at the proper place in the manuscript.”

We apologize for our mistake. The figure captions are in the proper place now, at the end of the document. Please see the “Figure legends” section in the manuscript.

“6. Figure 2 is not the self explanatory, required information should be provided for the same so that the figure could be understood well.”

Thank you for the opportunity to add more information to the HPLC chromatograms. Please see the “Figure legends” section in the manuscript.