Author's response to reviews

Title: In vitro antimicrobial activity of geopropolis produced by the stingless bee Melipona fasciculata Smith against oral pathogens and biofilm viability

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Author's response to reviews: see over
Response to the reviewer’s reports:

1) …Even though it has been known that propolis or geopropolis is non-toxic, the authors have to perform the cytotoxicity test against human oral tissue cells because the MBC value for S. mutans, from 6.25 mg/mL to 14.57 mg according to the fractions, was relatively high.

RESPONSE – Data from our laboratory have shown that topical application of geopropolis extracts on oral cavity has no toxic effects in pre-clinical assays in mice. Those results are included in other manuscript that deals with immunotoxicity to be submitted soon.
Version: 1  Date: 10 August 2011
Reviewer: VASSYA BANKOVA

Response to the reviewer’s reports:

1. Page 8, chemical characterization: the phytochemical methods for indicating the presence of triterpenes and alkaloids should be briefly described, the same way it was done for phenolics and flavonoids.

RESPONSE – We have described this section in more details (see pages 8 and 9)

2. Page 9, the last paragraph: the authors declare that HAE-3 contains no phenolic compounds but in Table 1 they report this extract to contain up to 51.2% phenolics. Please, clarify.

RESPONSE – This was a mistake. We revised the text (see page 10).

3. Table 2: it is not clear how many replicates were performed. Please, indicate and represent the results as mean value ± standard deviation.

RESPONSE – We performed duplicate assays in three distinct days. We included these information in the Material and Methods and also in table 2 (pages 6 and 23).
Response to the reviewer’s reports:

1) Page 2 (Abstract/Methods) – Ethanol 70%??? Chlorhexidine 0.12%??? (v/v or p/p)???
   Please, describe!

   RESPONSE – These information were clarified in the text

2) Page 2 (Abstract/Results) – The concentrations are confused: “…12.5 mg/mL for HAE-3…”
   and 14.5 mg/mL for chloroform…”. Please, verify and correct!

   RESPONSE – The sentence was rewritten

3) Page 5 (Background) – Line 2 to 6: Correct: “…Gram-positive...” and “…Gram-negative...”.
   Moreover, the sentence is confused and needs to be rewritten.

   RESPONSE – The sentence was rewritten and we have corrected “Gram-positive and Gram-negative” (last paragraph, pages 4 and 5)

4) Page 5 (Background) – Line 7 to 13: Some part of the paragraph is a resume of background sentences. Please, correct and rewrite the paragraph.

   RESPONSE – The paragraph was rewritten (page 5)

5) Page 5 (Methods) – Line 23 to 25: Please, explain better the proportion of propolis:ethanol 70% (v/v). Moreover, explain how were the propolis samples triturated? In a homogenizer?

   RESPONSE – We used a proportion of geopropolis/ethanol of 30/70 (w/v). The samples were triturated in a homogenizer (last paragraph, page 5)

6) Page 6 (Methods) – Line 1: Was the inorganic phase separated by vacuum? Please, rewrite the sentence explaining better.

   RESPONSE – The sentence was rewritten (last paragraph, page 5)

7) Page 6 (Methods) – Line 2 to 3: Please, describe the drying method of extracts. Moreover, write with more details the storage (in a refrigerator? What was the temperature?).

   RESPONSE – These information were included in the text (pages 5 and 6)

8) Page 8 (Methods) –
   8.1. Chemical- characterization section: This section needs more details. The methods used in this section need to be written with more details and more references are necessary about phenol content and total flavonoids content (Pharmacopoeias and so on).
RESPONSE – We have described this section in more details (see pages 8 and 9)

8.2. Why have the authors used 5% of aluminium chloride? Please, verify!

RESPONSE – We corrected this information for 2% (2nd paragraph, line 1, page 8)

8.3. Regarding the chemical aspects, the authors should have justified their option for phenols and total flavonoids content as a criterion “chemical characterization”. While it is true that Brazilian propolis is a rich source of phenolic substances, it is also true that most of such substances are not flavonoids. It is widely known that most phenolic substances from Brazilian propolis are prenylated phenylpropanoids, one of the most important being artepillin C (mainly for propolis from Apis mellifera).

RESPONSE – Although prenylated phenylpropanoids have been described in some studies as abundant components of “Brazilian propolis” produced by Apis, we did not performed a detailed chemical characterization of the geopropolis samples, but only a preliminary screening. Our option for total phenols and flavonoids was because the Brazilian legislation on propolis extracts describes them as some of the quality indicators of propolis extracts. A sentence on this subject was included in material and methods section (3rd paragraph, page 8).

8.4. The quantification of total flavonoids and phenolics was performed with calibration graph, but the results are not showed.

RESPONSE – The calibration equations both for gallic acid and quercetin were mentioned in materials and methods section (page 8 )

9) Page 10 (Discussion):
9.1. The results obtained for HAE-1 were not discussed!

RESPONSE – We have discussed this data in page 12, 3rd paragraph

9.2. The authors didn’t discuss their results comparing with others, including ethanolic extracts from Apis mellifera propolis. There are many studies that could be compared in the literature!

RESPONSE – We included a paragraph in the beginning of the discussion (pages 10)

10) Page 12 (Conclusions): The data shown the geopropolis ethanolic extracts (and some fractions) have activity against the microorganism tested. Moreover, just one extract (HAE-2) was tested about biofilm viability. Please, rewrite the conclusions!

RESPONSE – The conclusions were rewritten.