Reviewer's report

**Title:** Chemical Composition and antibacterial activity of Brazilian propolis type 6 and its fractions

**Version:** 1  **Date:** 4 April 2009

**Reviewer:** Zubaidah Rahim

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**ABSTRACT**

The authors did not clearly present the background and the methods employed in the study. This may be due to poor language used in the presentation.

The 1st sentence can be omitted. The authors did not clearly state how the antibacterial evaluation was carried out. Was it the HPLC, HRCG-FID and GC/MS fractions of the EEP, H-Fr and major fatty acids from propolis type 6 that were used in the evaluation?

In the Result and Discussion section of the abstract, the authors did not describe the 3 sub-fractions as 1, 2 and 3 though in the conclusion the authors mentioned sub-fraction 1.

The authors sub-fractionate the H-Fr using preparative HPLC. This makes the 1st sentence in the Method section misleading.

In the conclusion, it is clearer if the authors had described the 3 sub-fractions obtained from HPLC as sub-fractions 1, 2 and 3 in the Result and Discussion section.

The use of abbreviations

The authors used abbreviations without describing it first, e.g. HPLC, HRCG-FID and GC/MS. They were described in full only in the text.

**TEXT**

**Background**

The aim did not correspond with the aim in the Abstract. There was no mention of the elucidation of the chemical nature of bioactive compounds from Brazilian propolis type 6 in the Abstract.

**Methods**

**Section 1**

The method using TLC was not clearly described. What is being monitored? Is it correct to say that the components in EEP and H-Fr were separated using HPLC and identified using the reagents……

Though the P-Fr was isolated, it was not using in the subsequent study. What will
happen to it?

Section 2 and 3
What fractions do the authors meant in the 1st sentence, H-Fr and P-Fr?

Section 4
The authors would give a clearer picture by writing:
5 µL of the EEP was added to 5 mL of 96 % alcohol. The UV-VIS spectra of the mixture was determined and recorded. The experiment was repeated with 5 µL H-Fr. What is the purpose of this spectra?

Section 5
This was written wrongly as 1.5.
It would be clearer if the authors stated the fractions and sub-fractions in the second sentence. There is ambiguity as to the sub-fractions stated in the 5th and 6th sentences.

Section 6
This section need to be rewritten for clarity.
Controls for the antimicrobial assays
There was no mention of positive and negative (Blank) in all the MIC and MBC studies.
Statistical analysis
No mention of statistical analysis.
Results and Discussion
1st paragraph
The second sentence needs to include the word “respectively” at the end of the sentence.
The P-Fr was mentioned in the 1st sentence but subsequent discussion did not include it.
2nd paragraph
What is the significance of the UV-Vis spectral analysis?
3rd paragraph
It would be appropriate to use “3 peaks” instead of “3 chemical substances” in the paragraph.
4th paragraph
Write “The chemical constituents in the 3 peaks were ……” instead of “When the chemical ……”.
I would like to suggest the whole paragraph to be improved.
5th paragraph
The authors are right to say that fatty acids alone are not responsible for the antimicrobial activity observed for the propolis fractions. There are other factors
too. Perhaps the authors can carry out subsequent work to show this. This can be done by mixing other constituents identified in the PPE and H-Fr to the fatty acids and then do the antimicrobial assay using the mixture.

6th paragraph
Further studies mentioned by the authors are worth doing and the results obtained should be included in this paper. This will give a complement the second objective which is regarding the elucidation of the bioactive component.

Line 8
The spelling TCC should be TLC.

Line 12
The authors should state the references for ….."the literature suggest"…

Conclusion
The authors should not conclude the write-up by saying …"further studies"

Suggestions for improvement
The authors should make an attempt to use:

i. The component identified (polyprenylated benzophenone) in sub-fraction 1 for the antimicrobial assay.

ii. The mixture of fatty acids and the component in i) for the antimicrobial assay.

References
The authors did not write the references consistently. Please reference #10, #11, #13, #14, #16 and #20.

Table 1
No mention of the controls used in the study.
The results were given in values with a range. No statistical analysis.

Figure 1
The graph for EEP has no description of the X-axis. No explanation given for the 2 graphs.

Figure 2
The authors were not consistent in using the abbreviations EEP and H-Fr. In this figure, they used the abbreviation in full words.

Table III
The authors stated the positive control used but no mention of this in the Methods. The captions need to be improved for clarity.

NT= Not tested or ND= Not determined. Which one?
Other comments

After reading the manuscripts several times, I think it needs compulsory major revision. As it is the objective stated in the abstract does not complement the aim of the study.

The title does not actually convey the results obtained. The results obtained in the study suggested potential bioactive compound that may be responsible for the antimicrobial activity and this requires confirmation. The earlier comments include suggestions for improvement in this aspect.

The language used in the writing lacks clarity and hence needs professional help.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.