Reviewer's report

Title: Alternative preparation of propolis extracts: comparison of their composition and biological activities

Version: 2 Date: 12 November 2014

Reviewer: Cristina Marcucci

Reviewer's report:

1. Is the question posed by the authors well defined? Yes.
2. Are the methods appropriate and well described? Yes.
3. Are the data sound? Yes.
4. Does the manuscript adhere to the relevant standards for reporting and data deposition? No.
5. Are the discussion and conclusions well balanced and adequately supported by the data? No.
6. Are limitations of the work clearly stated? No.
7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished? Yes.
8. Do the title and abstract accurately convey what has been found? Yes.


Results: Total amount of phenolic compounds in extracts made in polyethylene glycol 400 (PEG) and water mixture or in PEG, olive oil and water mixture at 70°C was similar to that of ethanolic extract. Predominantly identified compounds were phenolic acids, which contribute ca. 40% of total radical scavenging activity.

Investigated nonethanolic extracts inhibited the growth and reproduction of all tested microorganisms (corrected form: microrganisms). Antimicrobial activity of some extracts was equal or exceeded the antimicrobial effect of ethanolic extract. Extracts made in pure water or oil only at room temperature, contained more than 5 – 10 fold lower amount of phenolic compounds, and demonstrated no antimicrobial activity.

Background

Propolis and its extracts have long been used for the prevention and treatment of a variety of diseases due to its antibacterial, antiviral, antifungal, antioxidant,
anesthetizing, cytostatic, anti-inflammatory, and immune-strengthening, hepato-protective (corrected form: hepatoprotective) effect, etc. [1, 2]. Among the many biological activities of propolis extracts, antimicrobial effects have been widely reported. Ethanolic extracts of propolis have been found to be effective against a broad range of bacteria, especially against gram-positive bacteria species [3].

Comments:
In many parts of the text is typed ml. Change to mL.

Results:
We have chosen a solvent content of 20 % PEG, because these pharmaceutical forms must be sterile (sterile filtration through a 0.22 micrometer pore membranes change to membranes) - higher PEG concentrations cause significantly more difficult filtration conditions.

Several studies demonstrated that propolis antibacterial properties were attributable to its high flavonoid content. Takaisi and Schilcher suggested that it is due to the action of the flavonone (change to flavanone) pinocembrin and the flavonol galangin, and caffeic acid phenethyl ester, whose action mechanism is based on the inhibition of bacterial RNA polymerase [25].

List of abbreviations
PEG: polyethylene glycol 400; DPPH: 1,1-Diphenyl-2-picrylhydrazyl radical; GAE: gallic acid equivalent; RE: rutin equivalent; W1: propolis extract made in water; W2: propolis extract made in PEG and water mixture; A1: propolis extract made in oil; A2: propolis extract made in oil, PEG and water mixture; EEP: propolis extract made in ethanol.

General comments:
In several parts of the text are underlined paragraphs. In addition, the text was typed in different spaces between lines. Check this format.

Figures:
Remove the Figures 2-5. Are not necessary to the understanding of the text. Make a table containing the information of the markers identified by HPLC in different extracts. For example:

<table>
<thead>
<tr>
<th>Compound (Rt*)</th>
<th>W1</th>
<th>W2</th>
<th>A1</th>
<th>A2</th>
<th>EEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeic acid (Rt)</td>
<td>+ +</td>
<td>- +</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>
Ferulic acid (Rt) + + + + ?
Galangin (Rt) - - - + ?
Kaempferol (Rt) - - - + ?
Naringenin (Rt) - - - + ?
Trans-p-coumaric acid (Rt) + + + + ?

*Rt: Retention time in minutes.

**Level of interest:** An article of outstanding merit and interest in its field

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

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

- Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this paper, either now or in the future? No.

- Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this paper, either now or in the future? No.

- Do you hold or are you currently applying for any patents relating to the content of the manuscript? Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript? No.

- Do you have any other financial competing interests? No.

- Do you have any non-financial competing interests in relation to this paper? No.