Reviewer’s report

Title: Anti-borreliae efficacy of selected organic oils and fatty acids

Version: 3 Date: 04 Nov 2018

Reviewer: Lidija Senerovic

Reviewer's report:

In this manuscript, the authors evaluate the effectiveness of a broad selection of organic oils and fatty acids against motile spirochetes, knob/round-shaped persisters and biofilm-like aggregates of Borrelia burgdorfei s.s. and Borrelia garinii. The study is well conducted providing promising results on the effectiveness of lipid-based in vitro treatments against Borrelia species, but the presentation of the results needs improvements before further consideration for publication.

Specific comments:

Abstract and Background

These two sections contain all the relevant information and no changes are required.

Material and methods

1. Pg5, Ln113: Were the liquid stocks also prepared in DMSO?

2. Pg7, Ln152: How many independent experiments were conducted? In the manuscript, it is only written: "All experiments were conducted in triplicate."

3. Pg8, Ln 168: Was the statistical analysis performed on technical or biological replicates? All the experiments should be performed at least three times independently.

Results

1. In Figure 1 the authors presented representative fluorescent images of 72h treated Borrelia species. I would suggest presenting images obtained after 24h of treatment (MBC50) in the main figure in order to be able to visualize kinetics of killing and be able to observe both live and dead cells. At 72h of treatment almost no cells could be detected, thus it is less informative than previous time points. Representative images of 48h and 72h treatment should be presented as supplementary material. Please also provide scale bars.
2. What do the error bars represent in Figure 2 and how were they calculated?

3. Why were the chemical structures of active compounds presented as parts of Figure 2 as the results with the compounds were also presented in Figure 1? I suggest complete removing of the chemical structures of these compounds as they are well known, otherwise, they can be part of the Supplementary material.

4. The authors used triple antibiotic treatment as a control in all experiments and these results should be provided in each figure.

5. The evaluation of anti-biofilm effects of the test lipids presented in Figure 3 was performed only using crystal violet staining and the effects, although statistically significant, are not prominent. It would be useful and more convincing if authors provide fluorescent images of treated biofilms. Treatment with triple antibiotics should be included. Please provide information on how were the error bars calculated and what do they represent.

Discussion

1. Discussion section contains extensive repetition of the results. The part starting at Pg10, Ln 230 and going up to Pg 12, Ln 265 contains the detailed presentation of the previously shown results and thus, should be condensed.

2. Some information was repetitively provided such as information on the effectiveness of daptomycin against Borrelia persisters (Pg10, Ln 225 and Pg12, Ln 276).

3. Although the statements regarding the antimicrobial activity of the oils and fatty acids were extensively cited, information on other activities was not referenced at all (Pg 13, Ln294-298). The missing references need to be provided.

Conclusion

The results of the study were properly summarised.

References

The citation list of the manuscript (79 in total) is too extensive and should be restricted. For many statements the number of references is unnecessarily large, particularly in Background and
Materials and methods sections, thus only the most relevant publications have to be selected. When citing methods one or two references would be enough (Pg. 6, Ln 134).

**Are the methods appropriate and well described?**  
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**  
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**  
If not, please explain in your comments to the authors.

Yes

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**  
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I recommend additional statistical review

**Quality of written English**  
Please indicate the quality of language in the manuscript:

Acceptable

**Declaration of competing interests**  
Please complete a declaration of competing interests, considering the following questions:

1. 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 manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. 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?
5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?

If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare that I have no competing interests.

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal.