Author’s response to reviews

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

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Author’s response to reviews:

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Dr. Anne Menard
BMC Complementary and Alternative Medicine

Manuscript: BCAM-D-18-00629R3

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

Dear Dr. Menard:

Thank you for accepting our manuscript BCAM-D-18-00629 (“Anti-borreliae efficacy of selected organic oils and fatty acids”) with major revision.

Attached please find the revised version of our manuscript in which we incorporated the suggested changes and modifications. We appreciate the time and attention paid to our manuscript by you and each reviewer. Per Editorial Office request, we ensured that all changes to the manuscript are indicated in the text by yellow highlighting.
Below are our specific responses to the editor’s and reviewers’ comments and suggestions:

Editor Comments:

1. Please change the Materials and Methods heading to Methods.

A. We applied the required change in the manuscript according to this specification.

2. The Availability of data and materials section refers to the raw data used in your study. As data was generated in your study, you cannot state "Not applicable". We strongly encourage all authors to share their raw data, either by providing it in a supplementary file or depositing it in a public repository and providing the details on how to access it in this section. If you do not wish to share your data, please clearly state this in this section along with a justification. Data availability statements can take one of the following forms (or a combination of more than one if required for multiple datasets).

A. We do wish to share our raw data and do not have any issues involved with the consent to publish our data. We exchanged the statement “Not applicable” with “All data generated or analyzed during this study are included in this published article”.

3. Please use the authors’ initials, not their full names, in the Competing Interests section.

A. We applied the required change in the manuscript according to this specification.

4. We would like to ask for you to provide more justification for the contributions of AN and MR, as currently they do not automatically qualify for authorship. Contribution to one aspect of the study, alone, does not usually justify authorship.

A. We applied the required changes in the manuscript according to this specification.

5. Please note, the role of the funding body in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript should be declared in the Funding section.

A. We applied the required change in the manuscript according to this specification.
6. Please provide a list of all the abbreviations used in the manuscript. This list should be placed just before the Declarations section. All abbreviations should still be defined in the text at first use.

A. We applied the required change in the manuscript according to this specification.

Reviewer #3:

Lidija Senerovic (Reviewer 3): 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 burgdorferi 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.

Thank you for reviewing our paper and identifying the areas of the manuscript that need further clarification.

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?

A. The liquid stocks were prepared in DMSO when there was a necessity to do that. We took the identical approach in performing our experiment as other research groups, e.g., Feng et al. Anti-Borrelia Activity of Essential Oils Frontiers in Medicine vol. 4, 2017: “Essential oils were added to BSK-H medium (or Borrelia sp. cultures) to form aqueous emulsion suspensions, followed immediately by serially diluting the essential oil suspensions to desired concentrations into Borrelia sp. cultures. Essential oils were also dissolved in organic solvent dimethyl sulfoxide (DMSO), followed by further serial dilutions to achieve desired dilutions.”
2. Pg7, Ln152: How many independent experiments were conducted? In the manuscript, it is only written: "All experiments were conducted in triplicate."

A. Our statement that "All experiments were conducted in triplicate" means exactly what Reviewer #3 implies, that there were three independent experiments performed and each one in three replicates.

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.

A. All the experiments were performed three times independently as statistical analysis requires taking such an approach in the experimental work. Since the reviewer pointed this out again we corrected the "All experiments were conducted in triplicate" statement in our manuscript to “All experiments were conducted three times independently and each one in three replicates.”

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.

A. The authors were asked by Reviewer #2 to provide representative images. Representative images are usually those that represent/visualize final results. This is an approach that other research groups in this field take presenting images of the experimental work after complete incubation period, e.g., Feng et al., or Sapi at al. Per the recommendation of Reviewer #2 we added the requested images of untreated (control) samples, and samples after treatment with eugenol and cinnamaldehyde (as representatives of the most effective oils), 13Z,16Z docosadienoic acid (as representative of the most effective fatty acid), and in addition triple combination of antibiotics (as a positive control), respectively. Since Reviewer #3 asked for images after 24 h we replaced the images after 72 h incubation period with the images after 24 h incubation period (and included scale bar). As the Reviewer #3 pointed out, images other than after 24 h incubation period are less informative; we prefer not to add any supplemental data to our manuscript.

2. What do the error bars represent in Figure 2 and how were they calculated?
A. The error bars represent ± SD (standard deviation) of a data set. Using Excel software they were calculated according to standard statistical analysis of experimental work from three replicates and by further averaging obtained numbers from three independent experiments. We also consulted the Department of Statistics at UC Davis, CA to validate our statistical analysis in the manuscript.

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.

A. Per the request of Reviewer #2, we added the chemical structures of the active compounds of the most promising lipids such as: eugenol (as active compound of bay leaf oil), cinnamaldehyde (as active compound of cassia oil), and 13Z,16Z docosadienoic acid, and they were incorporated in Figure 2. These structures were originally not incorporated in our manuscript. Since Reviewer #3 requested the authors to remove it, we comply with this request hoping that Reviewer #2 agrees with that.

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

A. We applied the required changes in the manuscript/all three figures according to this specification.

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.

A. Per the Reviewer’s recommendation we added the requested images of untreated (control) biofilm samples, and biofilm samples after treatment with eugenol and cinnamaldehyde (as representatives of the most effective oils), 13Z,16Z docosadienoic acid (as representative of the most effective fatty acid), and triple combination of antibiotics daptomycin+cefoperazone+doxycycline (as a positive control), respectively. Samples were stained with BacLight dye (live/dead staining). The merged images of biofilms are now incorporated in Figure 3. We would like to emphasize that these images were taken from random field/areas of the particular slides using merged green or red fluorescence to show viability of
biofilms, and included scale bars. We also included necessary information about this set of experiments in the Methods section of the manuscript. In regards to how the error bars were calculated and what they represent, we refer to our response/statement provided in point 2.

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.

A. Per the request of Reviewer #3, we condensed this part of our manuscript approximately 50% (from 347 characters to 197 characters).

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

A. We removed the repetitive statement on page 12 from our manuscript.

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.

A. Per the request of Reviewer #3, we added the missing references.

Conclusion:

The results of the study were properly summarized.

References:

1. 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).

A. We shortened the list of references from 79 to 69, which is a final number after including four additional citations requested by Reviewer #3 on page 13 of the manuscript.
We hope that the revised manuscript is acceptable for further review and acceptance. We look forward to your response.

Sincerely yours,

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