Author’s response to reviews

Title: Determinations of the effects of cinnamon bark fractions on Candida albicans and oral epithelial cells

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Author’s response to reviews:

REVIEWER 1 Specific comments
1. The abstract could show what MIC and MFC stand for, and its conclusion needs to be more objective. The abstract has been modified accordingly.
2. The manuscript does not explain how the two commercialised products were selected for this study. Perhaps previous studies testing the same products could be included in the background section. To the best of our knowledge, the two products used in our study have not been investigated yet. However, in the Background section, we justified our choice to work on cinnamon compounds (essential oil, proanthocyanidins) by the fact that numerous studies have reported on the therapeutic properties of cinnamon, although in a non-oral context. In the Discussion section, we discussed our results with previous studies that have used similar products.
3. Page 6 line 115 - Describe the number of dilutions/concentration of cinnamon fractions tested in the MIC assay. This information is now provided.
4. Page 6 line 119 - Clarify the time intervals of OD recording over the 24h of incubation. To determine the MIC, the optical density at 660 nm was recorded following a 24-h incubation that corresponds to the stationary growth phase. This information is now provided. We also extended the incubation to 48 h and found that it did not modify the results and conclusions.
5. It is not clear how a visual assessment was able to identify the inhibition of C. albicans growth, especially in a 96-well plate. We agree with this comment that a visual assessment of growth is a qualitative measure. Since we recorded growth by measuring the optical density at 660 nm, we deleted the visual assessment in the revised manuscript.
6. Page 6 line 127 - How was the ratio established? Please add references? This ratio is largely used by research groups working on antifungal agents; a reference has been added.
7. Page 8 line 154 - "24h"? Clarify how the Candida biofilm was grown in the biofilm viability test. Why was only cinnamon oil tested against mature biofilm?

More details are now provided. Cinnulin was not tested for microbicidal activity on C. albicans biofilms since this cinnamon fraction showed no antimicrobial effect on planktonic growth. A sentence has been added in the Results section.
8. Page 8 line 171 - What was the concentration of each cinnamon fraction tested? This information is now provided.
9. In Table 1, why was the MFC/MIC ratio for Cinnulin not calculated? Cinnulin did not exert any antimicrobial activity against C. albicans; therefore, no MFC/MIC ratio could be calculated.
10. The membrane permeability assay tested only the effect of cinnamon oil on C. albicans. How about the other natural products?

Again, since Cinnulin did not exert any antimicrobial activity against C. albicans, we did not initially tested its effect on membrane permeability. We performed the assay and as expected no effect was observed. A sentence has been added in the revised manuscript.

11. The
effect of the natural products on both Candida biofilm formation and mature biofilm would be essential to draw a conclusion regarding the potential prophylactic and therapeutic effect of Cinnamon bark oil and Cinnulin. However, the latter was not tested against 24h C. albicans biofilms. Consider the inclusion of this experiment in the manuscript. Since Cinnulin did not exert any antimicrobial activity against C. albicans, we did not test its ability to induce killing of a C. albicans biofilm. A sentence has been added in the revised manuscript. We performed the assay and as expected no effect was observed. 12. Page 16 line 352 - "These results suggest that Cinnulin PF® may be a promising anti-C. albicans agent because it specifically acts on biofilm formation but has no effect on growth." - Clarify why an effect on Candida growth is not desirable in an anti-Candida agent. We entirely agree with this reviewer that inhibiting the growth of C. albicans should be also considered as a desirable effect. The sentence has been modified accordingly. 13. It is not clear why cinnamaldehyde was tested in the study as there is not much discussion about the results of cinnamon bark oil and cinnamaldehyde alone. This is a good point; in the revised manuscript, we decided to deleted the only results on cinnamaldehyde. 14. The authors need to bring a more in-depth analysis of the impact of these natural products on the host and pathogen. For instance, discuss the role of IL-6 and IL-8 in the immune system and how the change in the cytokine profile caused by cinnamon products could influence the immune system against candidiasis. We fully agree with this comment and this point is now discussed in the Discussion section. 15. The study could try to conclude which Cinnamon-based natural product would be better against C. albicans considering their toxicity, antifungal, and anti-biofilm properties. It is rather difficult to identify which cinnamon fractions should be considered as the best one. As presented in our discussion, the fractions showed difference in their biological properties. We added a sentence in the Conclusion section stating that given their different actions on C. albicans, using them in combination may allow synergy.

REVIEWER 2
Specific comments
1. Title: reverse title, "effects" determination of … The title has been changed as suggested.
2. Abstract: grammar|: candida spp causes "not can" The abstract has been modified accordingly.
3. Background, line 66-67: rephrase the grammar The text has been modified accordingly.
4. Line 66: "accumulate in large amounts" biofilm are structures that formed by both dead and live Candida for protection and utilization of nutrients: the author should revise this statement. This sentence has been revised.
5. There is no Kirby Bauer technique approach that was used? In our study, we evaluated the antimicrobial properties of the cinnamon fractions using a broth microdilution assay that allows determination of both minimum inhibitory concentrations and minimum fungicidal concentrations. The Kirby-Bauer disk diffusion susceptibility test does not allow determining the fungicidal effect. Moreover, this technique is not recommended for essential oils since they are highly volatile. 6. Line 112-114: this is not McFarland's standards approach? Otherwise indicate? As suggested, we added the corresponding value of McFarland standards.
7. All methods/techniques are not referenced? We added several references in the Methods section.
8. Line 141: description on steps on biofilm structures formation is not yet explained? Rather than just culturing of the Candida spp on well and wash of the cultures after 24hrs. More details are now provided.
9. Justify why selected cytokines were used the rest excluded like cytokines like (IL)-4 or IL-12. We chose to focus on IL-6 and IL-8 since these two cytokines are secreted in high amounts by oral epithelial cells and have been associated with oral inflammation. Some text has been added in the Discussion section. Moreover, we performed an ELISA assay for determination of IL-4 and IL-12 and found that these two cytokines were not secreted by the GMSM-K epithelial cell line used in our study.