Author’s response to reviews

Title: Compounds from Olea europaea and Pistacia lentiscus inhibit oral microbial growth

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Author’s response to reviews:

The responses to the comments of the reviewers have been also attached as supplementary material.

Listed response to the Reviewers

Reviewer #3

The aim of this study is to investigate the antibacterial effect of a number of constituents from Olea europaea and Pistacia lentiscus. This is an interesting and relevant aim. My overall impression is that this is a methodologically well conducted study. My concerns regard the interpretation of the results.

1. Abstract
The abstract describes the manuscript well except for the conclusion. The conclusion in the abstract is not supported in the results. "...and could serve as efficient alternatives to antibiotics". There are two problems with this conclusion: A. the oral conditions usually treated with antibiotics are periapical lesions, severe periodontitis and sometime pericoronitis. There is nothing in this study showing that the tested substances would have any effect on these conditions. B. An antibacterial effect on planktonic bacteria is not the same as an effect on bacteria growing in a biofilm. As mentioned in the introduction, bacteria organized into a biofilm is much more resistant again antimicrobial substances.

Response

The conclusion sentence was modified as follows:

Overall, maslinic acid, 24Z-isomasticadienolic acid and oleanolic acid exerted the most significant inhibitory activity against the tested oral pathogens, especially streptococci and anaerobic oral microorganisms.

2. The conclusion at the end of the discussion is better even that one also needs to be changed.

Response

The end of the discussion was adjusted accordingly:

Overall, compounds from O. europaea and P. lentiscus such as maslinic acid and oleanolic acid were extremely effective against the tested oral pathogens, especially streptococci and anaerobic oral microorganisms. All tested extracts proved to be ineffective against the anerobic P. intermedia. Thus, future clinical studies should investigate the use of these natural antimicrobial agents in the treatment of caries- and periodontitis-related oral biofilms.

3. A detail - on line 73 and 74 it says that maslinic acid was the most effective compound against anaerobic pathogenes, it had no effect on Prevotella intermedia, this should be mentioned.

Response

The following sentence was added in the abstract:
All tested compounds showed no effects against Prevotella intermedia.

4. Introduction

The introduction is well written but I still think that it lacks some information about the reasons why these xx bacteria were selected. This is important for the understanding of the relevance of the study. This is my main concern with the introduction.

Response

Thank you for the question. The following information was added to the text:


5. I do have some minor points to:

Line 90 "of the most current therapeutic trends in medicine" I find this a strange expression, especially when it is supported by 13 years old reference.

Response

Thank you for this comment as well. A more recent citation (Newman DJ, Cragg GM. Natural Products as Sources of New Drugs from 1981 to 2014. J Nat Prod. 2016 Mar 25;79(3):629-61. doi: 10.1021/acs.jnatprod.5b01055) replaced the old one.
6. Line 115 "Recent years have seen the increase in chemotherapeutic intransigence of microbial biofilms," Has the resistance of biofilms really increased during recent years? In this section of the discussion there is a confusion between the increased resistance of biofilms per se and the existence of resistant bacteria in the biofilm. This should be sorted out.

Response

To avoid misunderstandings the sentence in the text was changed as follows:

In recent years the research on the chemotherapeutic intransigence of microbial biofilms, whose antibiotic resistance is 1000 times higher compared to planktonic bacterial cells, has been intensified [22].

7. Material and Methods

This section is very ambitious and meticulous and I hav only one concern; the samples were tested in duplicate. How were the results handled? Is it an average that is reported in the tables, if so there should be a variation. How big difference between the results was acceptable? They could hardly be identical. To chose the highest number is unusual, what is the rationale behind that?

Response

In each experiment all sample concentrations were tested in duplicate and no variations among the results for each tested concentration within one experiment could be observed. This is rather common, since we used an international standard method as mentioned in the manuscript. However, when the same experiment was repeated for the given sample concentrations, some variations were indeed detected for the MIC/MBC values. In the presence of variations among the yielded MIC/MBC values after the repetition of the experiments, the highest MIC/MBC values were chosen to eliminate false positive results. They latter may falsely depict an antimicrobial effect of the compounds which is much higher than their actual antimicrobial potential.

The following sentence was added to the text:

In the presence of variations within the yielded MIC/MBC values after the repetition of the experiments, the highest MIC/MBC values were listed to eliminate false positive results.
8. Discussion

This is the section that has the biggest potential for improvement. The discussion is quite long but it still doesn't address some of the important questions.

Response

The discussion was shortened.

The following paragraphs were removed:

«In a recent study, oxidative stress could be defeated by low concentrations of maslinic acid, while higher concentrations of the same agent induced apoptotic damages to melanoma cells [42].»

«In another study, the protective mechanism of oleocanthal against Alzheimer’s disease through the modulation of fibrillization of tau protein was highlighted [57].»

«Another tetracyclic triterpene, 24Z-isomasticadienonic acid possesses, through the suppression of 11β-hydroxysteroid dehydrogenase 1 anti-inflamatory, properties beneficial in diseases such as diabetes mellitus and disorders of lipid metabolism [66, 67].»

«In light of the increasing resistance to conventional antibiotics, numerous plant-derived antimicrobial agents give new hope for the discovery and application of novel phytopharmaceuticals with superior antimicrobial properties [68]. Additionally, the introduction of natural products will allow for the limited use of synthetic chemicals in dental industry and the reduction of their side-effects (cytotoxicity, tooth discoloration, nausea) [69, 70]. In the future, novel anti-infective medications should include not only the conventional antibiotics with limited effectiveness and lifespan but also small-molecule natural antimicrobial agents, which act in synergistically and thereby enhance the antibiotic performance [71-73]. This will allow for the eradication of microorganisms with low susceptibility to antimicrobials and the efficient antimicrobial treatment of sensitive patient groups such as immunocompromised or allergic subjects.»

9. In the very first line of the discussion the decreased susceptibility to antibiotics within oral biofilms is mentioned, which is correct but the problem is that this study has not investigated the effect on bacteria in a biofilm. The implications and importance of this discrepancy must be discussed.
Response

We appreciate this comment. Since we did not test the antimicrobial effects of plant extracts on biofilms, we removed the statement «In the light of the decreased susceptibility to antibiotics within oral biofilms» and the sentence remained as follows:

The present report introduced and screened eight antimicrobial compounds originating from O. europaea and P. lentiscus against nine representative oral pathogens.

10. It is good that some of the studies' compounds have a stronger effect on Gram negative bacteria as compared to Gram positive, since Gram negative bacteria are associated with periodontal disease, and Gram positive bacteria correlate with periodontal health. This should be discussed in this section of the Discussion.

Response

The following sentence was added to the discussion:

This fact is of high importance in oral infections, since Gram negative bacteria such as P. gingivalis are associated with periodontal disease while Gram positive microorganisms correlate with periodontal health (Dabdoub SM, Tsigrada AA, Kumar PS. Patient-specific analysis of periodontal and peri-implant microbiomes. J Dent Res. 2013 Dec;92(12 Suppl):168S-75S).

11. The discussion is more of a literature review, in my mind it could be shortened and focused on the findings in this study.

Response

The discussion was shortened (Please see response to Question 8).

12. Also, the conclusion could be condensed and more precise. None of the tested compounds can be considered extremely effective against P. intermedia.
The following sentence was added to the discussion:

However, oleanonic aldehyde as well as the other tested extracts proved to be ineffective against the anaerobic P. intermedia.

The following sentence was added to the conclusion:

All tested extracts proved to be ineffective against the anaerobic P. intermedia.

Response

Reviewer #4

The manuscript is well written and free of grammatical errors, in my opinion. It will attract a wide readership. However, I propose the following comments must be addressed to improve the manuscript.

1. The title should be rephrased possibly using the example such as "Constituents/Compounds of/from Olea europaea and Pistacia lentiscus inhibit oral microbial growth". The term "potent" as used must be done with measurements of potency in comparison to a reference antimicrobial agents such as IC50 or EC50 values. Without such determinations, the use of "potent" is not justified pharmacologically.

Response

The title was changed according to the suggestion of the reviewer as follows:

«Compounds from Olea europaea and Pistacia lentiscus inhibit oral microbial growth»

2. Authors have conducted that extracts from the plants have antimicrobial effects. The shift to compounds in these plant extracts is so sudden. The authors must provide information about the isolation of these compounds following their earlier work and make a case for the present study which is looking at the compounds from the plant extracts.

Response
The present work is a continuation of a research previously conducted: Karygianni L, Cecere M, Skaltsounis AL, Argyropoulou A, Hellwig E, Aligiannis N, Wittmer A: High-level antimicrobial efficacy of representative Mediterranean natural plant extracts against oral microorganisms. 2014, 2014:839019. In that work various plant extracts were examined for their antimicrobial efficacy against oral microorganisms. Some of the most promising plant extracts were tested further with the examination of their major compounds. The isolation of the compounds has already been published and it is described in the Methods section of the aforementioned manuscript.

The below sentence has been added: "Following our research on the antimicrobial efficacy of Mediterranean natural plant extracts, some of the most promising extracts were selected (Karygianni et al., 2014) and their major compounds were tested."