Author's response to reviews

Title: Inhibitory Activity of a Standardized Elderberry Liquid Extract Against Clinically-relevant Human Respiratory Bacterial Pathogens and Influenza A and B Viruses

Authors:

Christian Krawitz (Christian.Krawitz@mikrobio.med.uni-giessen.de)
Mobarak Abu Mraheil (Mobarak.Mraheil@mikrobio.med.uni-giessen.de)
Michael Stein (Michael.Stein@mikro.bio.uni-giessen.de)
Can Imirzalioglu (Can.Imirzalioglu@mikrobio.med.uni-giessen.de)
Eugen Domann (Eugen.Domann@mikrobio.med.uni-giessen.de)
Stephan Pleschka (Stephan.Pleschka@mikro.bio.uni-giessen.de)
Torsten Hain (Torsten.Hain@mikrobio.med.uni-giessen.de)

Version: 2 Date: 20 December 2010

Author's response to reviews: see over
Dear Sir/Madam,

please find attached a complete version of the revised manuscript entitled:

Inhibitory Activity of a Standardized Elderberry Liquid Extract Against Clinically-relevant Human Respiratory Bacterial Pathogens and Influenza A and B Viruses

for consideration as a publication in BMC Complementary and Alternative Medicine.

The amended version of the manuscript takes into consideration the recommendations made by the reviewers.

We hope that the manuscript is now in a form that is acceptable for publication BMC Complementary and Alternative Medicine. We thank the reviewers for the constructive comments and look forward to hearing your decision soon.

Yours sincerely

Torsten Hain and Stephan Pleschka
(On behalf of the authors)

Corresponding Authors:

Dr. Torsten Hain
Institute for Medical Microbiology, Justus-Liebig-University, Frankfurter Str. 107, D-35392 Giessen, Germany
Tel: +49 641 9941251; Fax: +49 641 9941259
E-mail: Torsten.Hain@mikrobio.med.uni-giessen.de

Prof. Dr. Stephan Pleschka
Institute for Medical Virology, Justus-Liebig-University, Frankfurter Str. 107, 35392 Giessen, Germany
Tel: +49 - 641 99 47750, Fax: +49 - 641 99 41209,
E-mail: Stephan.Pleschka@mikro.bio.uni-giessen.de
Response to the reviewer comments

Reviewer1: James Hudson
Reviewer's report:

major revisions:

1) The first section of Results (page 12, 1st para), and accompanying Table 1, are unnecessary, since the disc diffusion technique is not quantitative and can be misleading. The authors subsequently describe a more acceptable technique. I suggest replacing the disc diffusion section with a simple statement summarizing the result.

Response:

Amended as suggested.

As stated by the reviewer the disc diffusion technique is not quantitative and can be misleading. Therefore we have now focused the manuscript that a standardized elderberry liquid extract displays antimicrobial effects against the Gram-positive bacteria *Streptococcus pyogenes*, group C and G *Streptococci*, and the Gram-negative bacterium *Branhamella catarrhalis* in liquid cultures.

2) Tables 2 & 3, and Figure 1, report the same results in different formats. Consequently the tables are redundant and should be omitted.

Response:

Amended as suggested.

3) The final Results section on page 14 reports preliminary observations without data, and for which the authors cannot offer a satisfactory explanation. They should consider deleting this section since they add nothing to the manuscript.

Response:

Amended as suggested.

4) There seems to be some confusion between *Streptococcus* C and G strains, probably partly due to incorrect labelling in Fig 1, in which two growth curves are labelled *Streptococcus* Group G. Furthermore the data in Tables 2 and 3 indicate separate results for C and G. The authors need to check their data presentation, as well as their discussion and abstract, to ensure that their conclusions are correct.

Response:

Amended as suggested.

Similarly, in the Abstract they claim that the extract is active against MRSA and MSSA, which is not supported by the data presented.

Response:

Amended as suggested.

Minor revisions:

In Figure 2A, the middle panels should be labelled virus....."+". In my copy it
appears to be virus.... -. Also in Fig 2B there are no error bars.

Response:  
Amended as suggested.

Reviewer 2: mary ann lila  
Reviewer's report:  
Major Revisions:  
Essential need for a professional treatment of the 'chemistry' side of the paper, to  
parallel the 'bioassay' side. The latter is thorough, the former is scant.  
Minor Essential Revisions: all the rest.

REVIEW  
Title: Inhibitory Activity of a Standardized Elderberry Liquid Extract Against  
Clinically-relevant Human Respiratory Bacterial Pathogens and Influenza A and  
B Viruses  
Authors: Christian Krawitz, Mobarak Abu Mraheil, Michael Stein, Can  
Imirzalioglu, Eugen Domann, Stephan Pleschka and Torsten Hain  
Journal: BMC Complementary and Alternative Medicine

General comments|  
This contributed manuscript details some very straightforward, simple bioassay  
results to demonstrate the antimicrobial/antiviral properties of a standardized,  
diluted elderberry extract. While these properties have long been heralded for  
elderberry in general and for Rubini in particular, it is good to have some  
additional firm and comprehensive substantiation of these claims. In general, it is  
worthwhile to have stronger substantiation of properties attributed to members in  
the 'superfruits' category. The manuscript is very good in terms of antimicrobial  
and antivial assays.  
In terms of elderbery phytochemistry and bioactive compounds, it is very weak,  
the authors barely touched the chemistry. The report is not particularly innovative  
or does it offer any revealing hypotheses, however, it does provide  
corroborating and validation of these properties for elderberry, and does so in a  
quantitative manner that can be useful, plus it addresses the useful issue of  
simultaneous protection from bacterial and viral infections which can be  
concurrent.  

Specific comments|  
Abstract: lines 10-15. This is really not telling what the methods are, the text is  
exceedingly vague and not helpful. Stating objectives without giving a clue of  
methods. Be specific here and line out what this work is contributing by virtue of  
the alternative bioassays within. The quantitation, the evidence for concentration  
effects, the liquid versus solid – get this encapsulated here.

Response:  
The abstract was improved as recommended.  
The disc diffusion technique was removed from the text as recommended by  
reviewer 1.
Keywords: line 5. Here and in several other places in the manuscript, there is irregular ‘title case’ the word upper should not be capitalized here
Response: Amended as suggested.

Introduction:
Lines 3-5. Inappropriate wording
Delete the phrase “…and are also referred to as ‘antioxidants’”
Anthocyanins (most) do have antioxidant capacity, but it is inappropriate to word this in a sentence that implies synonymous terms.
Response: Amended as suggested.

Page 5, line 3-5
"The European black elder contains the flavonoids, quercetin and rutin. Anthocyanins of the berries, which are responsible for their dark coloration, were identified as cyanidin-3-glucosides [9]"
Authors need to rephrase this sentence and discuss more about elderberry bioactives. Elderberry contains different glycosidation forms of cyanidin. Cyanidin 3- glucoside is only one among other cyanidin glycosides.
Response: We have rephrased the sentence and discussed more about elderberry bioactives. To cite the additional information we included two new references:

“The European black elderberries are rich dietary sources of plant pigments and phenolic compounds. They contain the flavonols, quercetin-3-glucoside and quercetin-3-rutinoside, and a number of anthocyanins: a group of phenolic compounds responsible for the attractive red, purple, and violet colours of many fruits, flowers, vegetables, and also elderberries. The anthocyanins of elderberries were identified as cyanidin-3-sambubioside-5-glucoside, cyanidin-3,5-diglucoside, cyanidin-3-sambubioside, cyanidin-3-glucoside, cyanidin-3-rutinoside, pelargonidin-3-glucoside, and pelargonidin-3-sambubioside. The anthocyanins of elderberries are bioactive; for example, able to enhance the postprandial plasma antioxidant status of healthy humans (Netzel et al., 2005; Wu et al., 2004).”

References:

Methods:
p. 7 line 22 -- again, irregular title case. Brain Heart Infusion.
Response: Amended as suggested.
The elderberry-to-extract ratio of the product is 18:1 and the extract is standardized using membrane filtration to achieve a minimum anthocyanin concentration of 3.2% measured by HPLC."
The methods of standardization is not clear, need to write more details. Also need the HPLC quantification details and give a reference.

Response:
More details about the standardization and HPLC quantification as well as an appropriate reference method is now given in the text.

p. 8 line 18. again, irregular title case

Response:
Amended as suggested.

Results:
Lines 3-14. The timing to inhibition should be noted here and in the Table. Yes, it is in the Methods, however, timing is particularly critical to measured inhibition zones, and the authors are later making a point that the concentration/timing is even more striking in the liquid cultures, so the timing needs to be clear here.

Response:
The first paragraph concerning “disc diffusion” experiments was removed from the text as recommended by the reviewer 1. The topic about timing was amended as suggested within the manuscript in the paragraph "Antimicrobial activity of elderberry liquid extract in bacterial liquid cultures".
Tables 1-3 were also removed from the manuscript as recommended by reviewer 1.

p. 14 lines 8-19. This section is quite difficult to follow. Especially the unexplained final sentence.

Response:
This paragraph was removed from the text as recommended by the reviewer 1.

p. 23 Table 1 again, irregular title case, and, the TIMING is important here

Response:
Tables 1-3 were removed from the manuscript as recommended by reviewer 1.

p. 24 Table 2 CFU define – a Table is supposed to stand alone for easy interpretation

Response:
Tables 1-3 were removed from the manuscript as recommended by reviewer 1.

Reviewer3: Ladislav Kokoska
Reviewer's report:
The manuscript contains data on the antibacterial and antiviral effects of the extract from Sambucus nigra L. fruits. In my opinion, the effectiveness verification
of commercially available standardized product against clinically relevant pathogens is the main practical benefit of the study. It could be acceptable after extensive rewriting of several parts and implementation of certain data on methodology.

Major Compulsory Revisions
1. More detailed specification of the extract tested (especially extraction procedure used) and method of its characterization (HPLC conditions) should be provided in the METHODS section.

Response:
We have included more detailed specification of the extract tested and method of its characterization in the METHODS section as recommended by the reviewers.

2. Since clinical isolates of bacteria were used, it is important to provide data on their susceptibility to some reference antibiotic standard(s) [positive control(s)]. These data should be provided in Tables 1 and 3.

Response:
The recommended data are provided in Tables S1 as supplemental information.

3. The data on statistics (number of replicates and experiments) should be clearly indicated for each assay.

Response:
The data on statistics are added for each assay in the figure legends.

4. The DISCUSSION needs extensive rewriting (style of the first part of the section reminds rather description of results than discussion). The results achieved in the study should be compared more in detail with data previously published on antimicrobial and antiviral activities of S. nigra fruit extracts or compounds (e.g. Hearst et al., 2010; Manganelli et al., 2005). The RESULTS and DISCUSSION could be combined into a single section.

Response:
The discussion was carefully rewritten and the studies from Hearst et al., 2010 Nicholls et al., 2007 and Manganelli et al., 2005 were discussed and referenced in the manuscript.

Minor Essential Revisions
1. The data on geographical distribution, botany, and uses of S. nigra summarized in the first paragraph of BACKGROUND chapter should be supported by appropriate reference(s).

Response:
An appropriate reference (Germplasm Resource Information Network, United States Department of Agriculture) was added to the manuscript.

2. Since Table 2 repeats data showed in Figure 1, it can be excluded.

Response:
Amended as suggested.

3. Unification of data presented in different parts of the manuscript is also necessarily. For example, both agar diffusion and broth dilution tests are described in the METHODS section of the manuscript; however, the abstract mentions broth dilution tests only.

Response:
Amended as suggested. We have now focused the manuscript on broth dilution tests and have excluded the disc diffusion experiments from the manuscript as recommended by reviewer 1.

References

Response:
References are included yet.