Author’s response to reviews

Title: Efficacy of Moringa oleifera leaf powder as a hand-washing product: a cross-over controlled study among healthy volunteers.

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Author’s response to reviews: see over
Dear Dr. Prozenko,

Re: Efficacy of *Moringa oleifera* leaf powder as a hand-washing product: a cross-over controlled study among healthy volunteers. (revision)

We were pleased to receive your reply of 30/09/13 and are delighted to enclose our revised manuscript. We found the reviewers comments to be well balanced, insightful and extremely useful for the improvement of the article. We set out our response to specific comments in the text below.

Reviewer #1

Major Compulsory Revisions

1. Given the relatively small sample size did the authors do a power analysis prior commencing the study?

We followed the European Standards to test new hand antiseptics, which provide a full protocol with the different sample size and different testing steps required to demonstrate the efficacy of a new product. The same subjects were used to test both formulations in a cross-over manner. Therefore the reduction in bacterial counts can be compared for each individual, thus allowing a considerable reduction in sample size while at the same time retaining statistical power.

2. More information is needed about the powder used in the study. Was this a commercial product? If so what data was provided to confirm that it was 100%moringa leaf and not contaminated by other plant material? How was this product prepared? This is important as the processing may result in loss of active constituents and not reflect what might be seen in fresh leaves. This is also a point to be raised in the Discussion.

We used a commercial product made in Europe for which the manufacturing process followed European quality standards. The *Moringa oleifera* product comes as a dried powder. We believe that using a commercialized product which was prepared in a standardised way was more appropriate than buying or transporting the product ourselves. In the discussion (paragraph 7) we have
suggested the idea of testing fresh leaves and the potential advantages that use of fresh leaves could have in real settings.

Minor Essential Revisions

1. In the background there should be clarification that the discussion relates to microbial causes of diarrhoea rather than diarrhoea in general as there are non-microbial causes of diarrhoea.

We have clarified this confusion in the last paragraph of the background, when we stated that some evidence is found suggesting the potential “antibacterial” effect of Moringa oleifera leaf powder, and that the aim of this study was to test it using an in vivo study design. In the 1st paragraph of the background sections we also mentioned that diarrhoeal diseases can be caused by different microorganisms, not only bacteria.

Discretionary Revisions

• overall the paper is well written however there are a few places where the wording is a bit clumsy or prepositions are missing. These don’t detract from the meaning of the text but a careful proofread could address these issues and improve the flow of the text.

The text has been revised by an English speaker.

• The symbol for grams is g however is a couple of places (e.g. Table 2) it is shown as gr

. Corrections have been made in all text.

Reviewer #2

Minor revisions

1. Check that the conventions regarding botanical names are followed, with the botanical name being italicised and the genus name upper case, the species name lower case. There are a number of occasions where this is not adhered to, both in the methods and the discussion sections.

Corrections have been made in Methods and discussion sections.

2. I was unable to find an in-text reference to citation no 4 – Aiello et al (2008)

It is at the end of first paragraph in Background section.

Discretionary revisions
3. Does the method of handwashing discussed (application of a handful of powder, possibly wetted) seem to the authors as transferrable to a domestic setting? It would be useful to cover this in the Discussion.

This is a very useful comment. We have added a comment about this important issue in paragraph 6 of the Discussion section. At the end of the Discussion we also stated that this study only demonstrated the efficacy of *Moringa oleifera* under *in vivo* conditions, and that studies should be done to test the effectiveness of using this plant as a hand-hygiene product in real settings.

4. If the argument about the wet preparation being more effective because it has been extracted in water is being made, it would be useful to include discussion of the likelihood of water extraction of constituents with an antibacterial action (paragraph 2 of the discussion).

This argument is discussed in paragraph 3 of discussion. References 17-20 are supporting this argument.

5. I suggest paragraphs 5 and 6 of the discussion be removed – not relevant to this paper.

Paragraphs 5 and 6 have been removed.

Major revisions

There are a number of points of clarification required

Abstract

6. In the abstract, it is stated that the procedure was adapted from the protocol of the European Committee for Standardization EN 1500, but in the body of the work it is stated that the procedure is adapted from protocol EN 1499. Please clarify and correct.

This mistake has been corrected. The correct protocol is EN 1499. Both methods are very similar, and they are described in the discussion section.

Background

7. Last paragraph in this section states that, ‘There is a lot of anecdotal evidence supporting the effect of this plant as an antimicrobial agent and its use as a hand washing product.’ This statement requires citations, perhaps from ethnobotanical or anthropological literature.

We have added references to support the statement.

Methods
8. Under the section titled Moringa oleifera preparations – there is a statement that the substance tested was ‘leaf powder 100% natural from LUTOR’. What procedures were carried out to confirm the authenticity of the plant powder?

This product was manufactured in Europe subject to quality control and European trading standards legislation; therefore we believe we can trust the company’s description of the product (100% natural dried powder).

9. Under the section Hygienic hand washing procedure – the procedure for wet Moringa is unclear – clarify when the water was added to the plant material – was it added while the dry powder was in the hands, or prior to this?

We have clarified the application procedure in the Methods section, under the heading: Hygienic hand washing procedure. The water was added after the Moringa oleifera powder was applied to the participants’ hands.

10. In the discussion, (paragraph 3) it is suggested that the reason the wet Moringa preparation was more effective was due to water extracting active constituents. However it is not clear whether the powder was extracted with water, or whether water was used as a wetting agent.

The water was added on the top of the dried Moringa deposited previously on the volunteers’ hands. Water was used as a wetting agent. In the Discussion we suggested that wetted Moringa could be more efficacious in reducing bacteria, because water could help to extract some active ingredients from the plant which could facilitate the reduction of bacteria in hands. (See paragraph 3 in discussion). But we don’t mean to suggest by this that extraction should be done previously, we refer to the extractive effect of water at the moment of hand cleansing.

Discussion

11. Suggest that the Discussion is revised, both for logic (see below) and editing (eg ensuring that the same word is not repeated within a sentence).

We have read and rewritten some sections.

12. Paragraphs 7-9 of the discussion need to be redrafted. The authors suggest that the results did not meet the standards of EN 1499 but did meet those of EN 1500. This requires discussion of the differences between the two EU standards, and the applicability of each. The statement, ‘it can be argued that this product could also meet other standards used in different parts of the world’ needs further discussion with examples, and citations.

We have added discussion of all these issues in more detail in paragraph 5 of the Discussion section. We clarified the different uses of the European Standard methods (EN 1499 and EN1500) and also the differences between them. We note that products used in health care settings should meet one of
the regulatory standards (European or American), but this is not required of products for domestic use. We also refer to the variation in standards used in different parts of the world, and note that a product sometimes can pass one standard criterion but not another.

13. In addition, the statement ‘At present, it remains unknown which requirement is the right one or what decrease in microbial release is needed to produce a meaningful reduction in hand borne spread of healthcare associated pathogens’ is confusing and requires clarification, as not only does it seem to contradict the earlier citation of Curtis and Cairncross’ systematic review but also recent arguments by Elaine Larson (ie post the 1995 citation given here) are that handwashing is an integral part of hygiene practices that reduce illness. Please clarify.

With this sentence we do not question the efficacy of hand washing in disease prevention. Rather, we underline the uncertainty about the degree of reduction in bacteria on the hands of healthcare workers which is needed to achieve it. Is a $2\log_{10}$ or $3\log_{10}$ bacterial reduction factor, after health workers’ hand-washing enough to see a reduction of hand borne spread illness? Cairncross, Curtis and Larson consider the impact of hand washing on disease (measured by symptoms), whereas our arguments relate to causes of disease (measured by pathogen numbers).

We have added some clarification in paragraph 5 of the Discussion section.

Two final amendments come from our own revision; one in the Acknowledgement section, and the other stating that the first two authors contributed equally to the work.

We look forward to hearing from you regarding the result of this resubmission; we believe that addressing the reviewers’ comments has strengthened our submission. If we can provide any further information about our methods or results then please feel free to contact us at the address above.

Yours sincerely,

[Signatures]

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