Author's response to reviews

Title: Anti-microbial activities of pomegranate rind extracts: enhancement by cupric sulphate against clinical isolates of S. aureus, MRSA and PVL positive CA-MSSA

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Author's response to reviews: see over
Dear Natalie,

Thank you for the opportunity to submit a revised version of the above paper. We thank the referees for their helpful comments which we have addressed as detailed below. We have also modified the Competing Interests statement in line with your email.

We trust that the manuscript will now be acceptable for publication in BMC Complementary and Alternative Medicine.

Kind regards
Declan

The changes are as follows:

1. Revise the 'Competing Interests' section of your manuscript so that it states: 'The study was in part funded by Nature Therapeutics Ltd. The authors declare that they have no other competing interests.'

This has been completed.

Reviewer's report:
The manuscript describes the antimicrobial activities of pomegranate rind extracts and its cupric salts enhancement against clinical isolates of Staphylococcus aureus, MRSA, and PVL-positive CA-MSSA. The research was properly done and represents the first report of the efficacy of a pomegranate-derived natural product mixture against clinical PVL-positive CA-MSSA isolates.

Minor Essential Revisions:
1. Although "meticillin" is a recognized term, the authors may consider to change this to "methicillin" in order to be in line with the consistent use of the latter term in the cited references.
We have changed meticillin to the USAN nomenclature of methicillin. We had used the name meticillin in line with the BAN/INN nomenclature system.

2. The term "natural product" is more often used to describe a pure compound derived from natural sources, e.g., ellagic acid from pomegranate rind. The authors used this term to describe a mixture of secondary metabolites present in an aqueous extract of dried rind - please reconsider.

We have changed ‘natural products’ to ‘extracts’

3. The authors need to be consistent when using the term "et al."

We have changed these.

4. Reference 19 is a repetition of reference 9.

This has been rectified.

5. The authors need to consistently insert spaces between a numeral and the appropriate unit, e.g., 0.2 mm, not 0.2mm, also the abbreviation for milliliter is mL, not ml.

We have made the necessary changes.

6. The authors need to refrain from using a term like iron sulphate. It is either Fe(II) or Fe(III), i.e., ferrous- or ferric- sulphate. In the same paragraph where this appears, several corrections need to be made (Stewart and colleges, etc.), and the sentence starting with, A recent study ..., has to be reworded.

We have changed this.

7. The references section needs thorough revision, e.g., Staphylococcus aureus should be consistently italicized, manuscript titles should only be capitalized at the beginning, ref. 9 - please correct antibacterial "activates", ref. 20 - remove the periods in the journal abbreviations, and ref, 24- is there an abbreviation for "Veterinary Microbiology"?

We have changed this section.

Level of interest: An article of importance in its field
Quality of written English: Needs some language corrections before being published

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Reviewer's report:

Minor Essential Revisions:
The submitted manuscript displays an innovative approach to screening for antimicrobial activity in pomegranate extracts by combining the extracts with copper salts. The aim of the study is clearly presented against the background of current knowledge, which provides rationale for the study. The paper is well-written and grammatically there are few noticeable errors.

In line 15 on page 4, “affect” should be replaced by “effect”.
We have changed this.

On line 12 of page 7 “turbidly” should be replaced by “turbidity
We have changed this.

In line 23 of page 7, “of” should be inserted between “Addition” and “the”.
We have changed this.

In the Results, the first line should be rewritten.
We have changed this.

In line 2 of page 10, “colleges” should be replaced by “colleagues”.
We have changed this.

In the Discussion the sentence spanning lines 3-6 on page 10 should be rewritten.
We have changed this.

In the Methods, more information should be given on how the isolates were identified, and by whom. Were the bacteria classified as resistant according to their in vitro antibiotic susceptibility profile, or by response to treatment in a clinical setting? Were reference cultures stored for future comparison studies?
We have added more information. Regarding storage of the bacteria in the method section it states the bacteria were frozen at -80°C.

In the Discussion it should be noted that the MIC values obtained in this study are relatively high compared to those reported for many other natural products. Are these MIC values sufficiently relevant to justify further studies?
We have added a comment on this is the discussion.
In Table 2, a legend could be inserted to describe what the two MIC values are for the column PRE/CuSO4.

**We have added a description to the legend.**

**Level of interest:** An article whose findings are important to those with closely related research interests

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**Reviewer's report:**

Except the first paragraph, the entire introduction is exactly same as the published article including the same references.

**We have changed the small overlap in material in the introduction.**

Page 3, line no. 15 the quoted reference No. 9 and in methods page 5 line no. 7 the reference No. 19, both are same. Now you see in the published article in methods the reference quoted in 19. But this 19 refers to Stewart et al. 1998 while in the present paper Reference 19 refers to Mathabe et al. 2006. Explain

**We have changed this.**

Page 4 line 17 – Gram negative laboratory strain of bacteria – explain

We are unsure what reviewers question is “Gram negative laboratory strains of bacteria (Pseudomonas aeruginosa, Proteus mirabilis and Escherichia coli)” the bacteria within the brackets are all Gram negative bacteria.

Page 4 line 23 – PRE/metal ions – PRE and metal ion slash means or not and

**We have used this abbreviation in our recent previous published paper in this journal. It would be confusing to change it now. We have defined it in the last paragraph of the introduction.**

Page 4 line 24 stabilizing agent – which?

**We have added in brackets vitamin C.**

Page 5 line No. 18 isolates were passed twice on nutrient agar – Please state the specificity and provide the reference for the same

The phrase is passaged twice. This is a standard microbiological technique for reviving isolates that have come from a -80°C freezer.
This is a standard microbiological technique.

Page 5 line 23 – Antibiotic sensitivity testing – Antibiotics were procured from which source give details.

We have added source.

Also check the spelling of meticillin in entire document and check the spelling of gentamcin.

Under International Union of Pure and Applied Chemistry nomenclature gentamicin is the correct and acceptable spelling of this drug. We have changed the spelling of meticillin from the BAN/INN nomenclature to the USAN nomenclature of methicillin.

Page 6 line 6 – Antimicrobial activity of PRE with the addition of metal salts – metal salts is wrong it should have been cupric salt

We have corrected this throughout.

Page 6 – line 12 – metal salts (FeSO4, CuSO4)- FeSO4 is wrong

We have corrected this.

Page 6 line 18 – Lambda buffer – give details of composition or make

We have added a reference.

Page 7 line 1 – metal salts should have been cupric salt

We have corrected this.

Page 7 line 1, 2, and 3 not clear about the concentrations.

We are unsure what review is asking, as the concentrations are stated in brackets.

Page 7 line 5 – MIC determination of PRE and CuSo4 – no reference is cited for the method.

This is a standard technique.

Page 7 line 7 - in a concentration of 800 mg/ml . I think there is some error

There is no error, 50uL of ISB to 500uL of Pre (800mg/mL), takes the concentration to 400mg/mL. The addition of bacterial suspension takes the concentration to 200mg/mL.
Page 7 line 7 – The description of the plates is not mentioned properly.

We have added plate type and supplier information.

Page 7 line 8 – what is the make of Iso-Sensitest broth? Please mention

We have added the supplier Oxoid.

Page 7 - How MIC is calculated? No standard reference is given

This is a standard technique.

Page 7 – line 20 – PRE/CuSO4 combination - What does this means PRE and CuSO4 or do you mean PRE or CuSO4. The use of slash is wrong here.

Please see previous comments regarding slash.

Page 7 – line 23 – wrong English

We have revised this.

Page 8 line 5 – resistant to four of the eight antibiotics tested

We have changed to add ”to”.

Page 8 line 8 – addition of metal salts – it should have been addition of cupric Salt

We have changed it to cupric.

Page 8 – English has to be improved. Use of negating (line 14) and negates (line 22) is not proper

We have changed this.

Page 9 – line 7 –again the use of metal salts is wrong and why data is not shown for this work?

Data does not show a difference and it was felt of little interest.

Page 9 line 16 – it should have been Table 2 – not Table 1. The results are not properly discussed.

We have made appropriate changes.

Page 9 – line 22 – discussion part – badly written.
We have addressed this.

Page 10 line 16 – blank sample – What is blank sample?

We have added in the methods that lambda buffer is used as blank.

The use of metal salts, PRE/CuSO4, is wrongly done in the entire manuscript and it becomes very difficult to understand what the author wants to say.

Please see previous comments on this. These have been changed throughout the document.

Now for references section – Most badly and carelessly written section.

These have been corrected.

Over all comments:
The question posed by the author is not well defined.
The methods are appropriate but not well described.
The figures are not explained properly. They are not self explanatory
Use of metal salts and in one place FeSO4 is also included instead of cupric salt shows the carelessness of the author.
Most of the introduction and discussion is repetitive of the previous work.
The quoted references are wrong in some of the places.
The results obtained are moderate.
Overall it is not to the high standard of the journal (in my opinion) hence it is rejected on the basis it is scientifically not sound, but the editor can take the final decision.

We have corrected the issues with references and metal salts/Fe. We note that the other reviewers’ comments include:

- The research was properly done
- ….represents the first report of the efficacy of a pomegranate-derived natural product …..
- The aim of the study is clearly presented against a background of current knowledge…..
- The paper is well-written and grammatically there are few noticeable errors.