Author's response to reviews

Title: In vitro antimicrobial activity of plants used in traditional medicine in Gurage and Silti Zones, south central Ethiopia.

Authors:

Alemtshay Teka (AlemtshayTeka.Sahile@UGent.be)
Johana Rondevaldova (rondevaldova@ftz.czu.cz)
Zemede Asfaw (zasfaw49@yahoo.com)
Sebsebe Demissew (sebseb.demissew@gmail.com)
Patrick Van Damme (Patrick.VanDamme@UGent.be)
Ladislav Kokoska (kokoska@ftz.czu.cz)

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Author's response to reviews: see over
Dear Dr. Rowles,

**RE**: Submission of revised version of manuscript (MS: 5679874201702748)

We would like to thank the reviewers for their constructive comments and suggestions. We have addressed all issues indicated in the review report, and believe that the revised version can meet the journal publication requirements.

Kind regards,
Alemtshay Teka
Reviewer comments and responses

Reviewer 1: (Ambrose Okem)

Reviewer's report:

Major revision

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

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests: I declare that I have no competing interests

RESPONSE: We greatly appreciate the reviewer’s efforts to carefully review the paper. As suggested by the reviewer’s, we have added all the comments and revised the manuscript accordingly.

COMMENT #1 What concentration is regarded as good, moderate and poor antimicrobial activity? The most acceptable concentration is less than 100 µg/mL

RESPONSE: We agree that the most acceptable concentration is less than 100 µg/ml. As described in Cos et al. [28] that relevant and selective activity relates to IC50 values below 100 µg/ml for extracts. In this study we used 80% inhibitory concentration (IC80) of the growth control therefore compared to IC50 our values can be higher. Moreover experiments with MICs higher than 1 mg/ml for extracts should be avoided from further research as recommended by Rios and Recio (2005), in which case all our results are below 1 mg/ml.


COMMENT #2 Line 5: The authors should provide the GPS location of where the investigated plant species were collected

RESPONSE: We agree with the reviewer comment. We added GPS locations.
COMMENT #3 Line 6: The authors should provide the voucher specimen numbers for the investigated plant species
RESPONSE: We agree with the reviewer comment. We included the voucher specimen numbers.

COMMENT #4 Line 8: The authors should provide names of the senior plant taxonomists that identified plants and they should be acknowledged in the acknowledgement.
RESPONSE: Thank you for the comments. The name of experts identified and confirmed the identification are mentioned in the material and method. The staff members of the National Herbarium (ETH) are also acknowledged.

COMMENT #5 Line 13: The authors claimed that plants species were selected based on the under-research species however, a glance at literature showed that most of the selected plants species haven extensively investigated. I suggest the authors’ double check literature on the investigated plants species.
RESPONSE: The sentence the reviewer mentioned was added to show that some of the plants are under researched (such as Polygala sadebeckiana and Thunbergia ruspoli). The text has been revised.

COMMENT #6: Results
The authors reported antimicrobial activity for only 5 plant species as shown in Table 2. I suggest the authors include results for the other plant species, otherwise reasons for excluding results for other plants species must be clearly stated.
RESPONSE: As suggested, we added all the results report in Table 2.

COMMENT #7
Line 29: [16, 17], please delete “17” because this literature did not contain any information on Candida albicans
RESPONSE: We apologize for the error. Citation “17” is re-arranged properly.
Reviewer 2 (Patricia Combarros-Fuertes)

Reviewer's report
The manuscript titled “In vitro antimicrobial activity of plants used in traditional medicine in Gurage and Silti Zones, south central Ethiopia” written by Alemtshay Teka et al. deals with a very interesting topic. This research work evaluates, in vitro, the antimicrobial activity of different plant extracts in order to look for efficient and necessary alternatives to modern medicine to treat infectious diseases produced by microorganisms which are resistant to drugs currently used to treat them. The results derived from this research study could offer information from a scientific point of view and validate the traditional use of these plants. However, the article has some weaknesses which make difficult its publication.

I recommend to reformulate some parts of the article and to include some aspects, such as a simple statistical analysis, and after a major revision, I think that would be acceptable for publishing in BMC Complementary and Alternative Medicine.

RESPONSE: We greatly appreciate the reviewer’s efforts to carefully review the paper. As suggested by the reviewer’s, we have added all the comments and revised the manuscript accordingly.

Major Compulsory Revisions

COMMENT #1: The quality of written English is not good enough. This fact make difficult to understand what the authors want to say in some occasions. I would advise revising in general the English language on the all manuscript and doing some language corrections.

RESPONSE: Manuscript was revised on language quality by Dr. Wouter Vanhove (Faculty of Bio-Science Engineering, Ghent University) and Prof. Townsend Peterson (University of Kansas Biodiversity Institute)

COMMENT #2: I recommend doing a statistical analysis (a simple one is enough). A scientific work needs a statistical analysis to validate the study and make the results obtained more robust. I am not a specialist in statistic but in my opinion, applying a one-way ANOVA test or a Kruskal Wallis test (depending on if your data have or not homogeneity in variances) is enough to confirm the differences observed between


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the microorganisms tested treated with the same plant extract. The homogeneity of variances could be assessed with a Levene test (p>0.05 the samples have homogeneity of variances; p #0.05 the samples don’t have homogeneity of variances). Apply these tests when possible and adapt your “Results” and “Discussion” sections taking into account the results of this statistical analysis. Standard deviations of the data from the different experiments should be added too.

**RESPONSE:**

We completely agree that statistical analysis is important for validation but in present study the use of different statistics is not required since the objective of our study was to find the most effective plant extract against the tested microbes. Evaluation of results has been made according to the international standard, CLSI (Clinical and Laboratory Standards Institute), Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically: Approved Standard, 8th ed. (Document M7-A8), Wayne, PA, 2009 and we have a number of similar papers [e.g. Novy et al (2011) *In vitro* synergistic effects of baicalin with oxytetracycline and tetracycline against *Staphylococcus aureus*. J Antimicrob Chemother 66: 1298–300; Lulekal et al (2014). Antimicrobial activity of traditional medicinal plants from Ankober District, North Shewa Zone, Amhara Region, Ethiopia. Pharmaceutical Biology, 52: (5) 614–620; Carranza et al. (2015). Antibacterial activity of native California medicinal plant extracts isolated from *Rhamnus californica* and *Umbellularia californica*. Annals of Clinical Microbiology and Antimicrobials, 14:29)]. Statistics is described by the sentence “Results reported in this study are expressed as the mode of MICs obtained from three independent experiments that were assayed in triplicate.” The mode is the value that appears most often in a set of data and allowed microbiological error is ± one concentration e.g. of calculation is 4, 8, 8 (first experiment) and 4, 8, 16 (second), and 8, 8, 16 (third). The mode is 8. We believe that this way of standard evaluation of antimicrobial test makes use of others statistics inappropriate.

**COMMENT #3:** Please rewrite the Discussion because I think that the ideas are a little bit disconnected between them. For example the second paragraph stated some ideas individually but all of them are connected, rewrite them in order to show this connection.
RESPONSE: Thank you for this direction. We revised discussion part for language quality.

Minor Essential Revisions

COMMENT #1: Background- Paragraph 2 (almost at the end): “medicinal uses for the treatment of infectious diseases…” Please include one “s”
RESPONSE: Thank you. Edited as suggested.

COMMENT #2: Material and methods- In “Assessment of minimum inhibitory concentrations (MICs)”: In the middle of the paragraph “Microorganisms´growth” and “microrganisms´growth”. Please delete the apostrophe in both cases.
RESPONSE: Edited as suggested.

COMMENT #3: Results- Please delete the apostrophe and the “s”, here it no make sense.
RESPONSE: Edited as suggested.

COMMENT #4: References- “Giday” and “Zay people” Please correct these mistakes.
RESPONSE: Well taken, typographic errors corrected

Discretionary Revisions

COMMENT #1: Abstract-
In “Methods”: I think that in this section is better to clarify that the extracts were obtained from different parts of the plant depending on the kind of plant.
In “Results” (almost at the end): “…at a higher MIC value…” it would not be "at a lower MIC value"? Because the MIC value with your extract is between 128 and 256 µg/mL and with oxacillin is 512 µg/mL. You have a higher antibacterial activity because you have a lower MIC.
In “Conclusions”: I think is better to put “The study revealed in vitro antibacterial activity…….”
RESPONSE: Reviewer comments are well taken. Text are clarified and edited as suggested.
COMMENT #2: Background- Paragraph 1: you talk about “antibiotic-resistant” or “antibiotic”. As you worked with bacteria and one yeast (C. albicans) strains, I think it would be better to talk in general and not to focus only on bacteria (is only a suggestion).
RESPONSE: Edited as suggested in first paragraph.

Paragraph 3: I think it would be appropriated to make a comment, in general, on how these plants are prepared and how they are applied in traditional medicine.
RESPONSE: Reviewer comments are well taken. We added a column in Table 1 that illustrates route of administration.

COMMENT #3: Material and methods-
Comment: In “Selection plants”: What do you mean exactly with the term “Voucher specimens” I have already seen it in other publications of the same field but I do not completely understand. If you worked with different parts of the different plants please, explain how did you “press” them (conditions, how did you process them…)
RESPONSE: Voucher specimens are the pressed plant samples deposited in the National Herbarium (ETH) for future reference. The specimens are pressed using a plant press. But for laboratory analysis, we used air dried plant parts (not pressed). We explained this point in “Preparation of plant extracts” section.

Comment: I would change “The plants were identified ...Ethiopia” by “The plants were identified and then authenticated by specialized plant taxonomists in the Department of Plant Biology and Biodiversity Management, Addis Ababa University, Ethiopia”
RESPONSE: Thank you for this point. The statement is revised.

Comment: In “Microorganisms”: I advise you to explain in more detail which microorganisms were used with which extract because not all of them were tested with all the extracts. You explain something in “Results” but in my opinion you should clarify here.
**RESPONSE:** Thank you for this point. We agree with the reviewer point with regard to explaining which microorganisms were tested with which extract. However, we think that grouping the microorganisms used in the methods section could create confusion hence it is made so following the result obtained in the first test (result section Table 1).

**Comment:** In “Assessment of minimum inhibitory concentrations (MICs)”: How did you do your “full growth control”?

**RESPONSE:** We obtain a full growth control without adding plant extract or antibiotics. We edited and clarified this point in the text.

**COMMENT #4: Results**

**Comment:** I think that you should to talk on all the results even though some extracts were not active. A negative result is also a result. At the end you can conclude that more studies are needed to confirm these results if you do not want to reject its possible medicinal value.

**RESPONSE:** As suggested, we added all the results report in Table 2.

**Comment:** Paragraph 2. “Moreover, this extract…than oxacillin (512 µg/ml)” Is not there some mistakes? “Moreover, this extract showed activity at a similar or even lower MIC value for the test against *S. aureus* ATCC 33591, ATCC 33592, SA3 and SA5 strains (128-256 µg/ml) than oxacillin (256-512 µg/ml)”.

**RESPONSE:** Reviewer comments are well taken. The statement has been edited. In order to clarify the text ‘Lower MIC value’ is changed to ‘higher antibacterial activity’

**COMMENT #5: Discussion**

The same comment. I think you should to discuss all the results.

**RESPONSE:** Thank you for the suggestion.