Author's response to reviews

Title: Antimicrobial activity and cytotoxicity of the ethanol extract, fractions and eight compounds isolated from Eriosema robustum (Fabaceae)

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Author's response to reviews: see over
Respond to Reviews and Editor Comments

(Our response is highlighted in green)

Reviewer 1

The isolation of compounds from Eriosema robustum is not novel, as these have been previously reported by the same research group, but certainly the antimicrobial and cytotoxicity values make a valid contribution to the scientific field. The work thus presented here makes an interesting and valid addition to furthering the knowledge of medicinal plants and their respective compounds.

There are some aspects that are worth clarifying before final acceptance of the publication though. These are the following;

1.) Defining poor (or weak), moderate and good activity
This aspect is particularly confusing in the abstract where “weak antifungal activity (MIC 65 µg/ml) was obtained with some compounds” Later the authors refer to “highest antifungal activity (average MIC 88 µg/ml)” and “most active constituent against bacteria (average MIC 55 µg/ml). Due to the inherent doubling dilution of MIC testing, all these values are very similar and thus, I would recommend they be given similar classifications of activity. Furthermore, the authors often refer to activities of 65/63 µg/ml as been poor. I think the authors are being far too modest and perhaps they should consider this activity moderate to good in light of publication by Gibbons, 2004 who suggested that isolated compounds with antimicrobial activities of 64–100 µg/ml are accepted as having clinical relevance. It is very unusual to see a group of researchers that rather underplay their results rather than inflate them, so this research group should be commended for their conservative approach to reporting results. I would suggest that a classification (MIC range) of what is considered poor, moderate and excellent activity should be given, which will clarify this issue. The classification should be based on what has been previously reported in literature. The results can be discussed in light of these ranges.

We can see that the way we formulated the abstract may be confusing and this has been addressed. Changes are indicated with green highlight. We have also made some comments on what should be considered significant with some references.

2.) Background to study
Instead of an introductory paragraph dealing with medicinal plants as a predominant primary healthcare option, I would have preferred to have seen more of a background on the traditional medicinal uses of the study plant Eriosema robustum. When searching for background information on this plant species, very little could be found. I think a more valuable approach would be to give an extensive overview of the medicinal properties of this plant rather than an over of general medicinal plant use which is well known and frequently cited in numerous ethnopharmacological publications.

Thank you for the suggestion, but we reported all the information we found in the traditional medicinal uses of this plant.
3.) Care needs to be taken on a few typos and grammar e.g. “Thus, man uses his environment and the resources of nature to combat diseases that afflict him.” Also “The colourless salt of tetrazolium acts as an electron acceptor and was reduced to a red colour formazan product biologically by active organisms”. Check spacing between headings. DMSO as an abbreviation should be written out in full first time of use. Reference to Table rather than Tables (first line pg. 9). Avoid using words referring to text “above” see top pg. 11

 Corrections have been made on some sentences and words as mentioned. Spacing between headings are now regular and DMSO is written in full in its first time of use in the manuscript.

4.) Methods
Please specify where clinical microbial strains were sourced.
Reading MIC’s. After adding INT, the plates were left 1-2 hrs (bacteria) or 16-24 hr for fungi. One can see differences between the MIC’s been reported for these two times. The NCCLS guidelines for reporting MIC values states that they should be comparatively read with a control (pathogen without inhibitor). When wells with INT become visible, then all results become feasible to read. I think reading results after one hour incubation is not sufficient time for a colour reaction to take place. I know this method has been published extensively previously but I urge the investigators to go with reading results when exposed to longer time frames. In this case, report results only after 2 hrs./24 hrs. where relevant. By reporting both sets of results, it’ gets confusing. Which is correct? This will also avoid the comparison of results where a result of 1 hr. from a pathogen gets compared with the results of 2hrs from another pathogen.

Specification of the source of the clinical microbial strains is now provided in the manuscript.
The reading of the MIC’s after adding INT for bacteria is considered after 2h. change has been made in the manuscript.

5.) Results
Please be careful with reference to the word “kill” mid pg. 10. An MIC analysis only measures inhibitory activity.
An interesting approach to the speculation of synergy between compounds would have been to combine the compounds and determine MICs on the combination and determine the interactive index.
Table 1 needs to be changed with respect to presenting the data. Further to previous comments suggesting the presenting of only latest MIC results, the crude extract and fractions are given in mg/ml. As the table is presented here (see title) activities are presented in µg which is inaccurate.
Why were no end points determined for gentamycin.
Please include DMSO control results somewhere. Also final concentrations of DMSO should be specified.

The word “kill” has been change in the manuscript to “inhibit”
Thank you for the suggestion about the approach of the synergy between compounds, it is also possible to check the same effect from the total activity by comparing result of extract, fractions and isolated compounds.
Modifications have been done in Table 1 as requested from previous comments. The results of extract and fractions are reported in mg/ml and those of the compounds in μg/ml. Gentamycin is a well known standard antibiotic. In the result, none of our samples was more active than this reference and we think there was no need for us to determine its end points. DMSO control results have been included in Table 1 as well as its final concentration.

6.) Cytotoxicity
Reference to the cytotoxicity findings should be given in the conclusion and possibly some validation also given in the introduction.

Some sentences have been added in the introduction and conclusion regarding this suggestion.

Reviewer 2

There is no need to indicate the family of plant in the title.

We think that it is important to mention the name of the family of the plant in the title if other readers are not acquainted with the genus. 1H and 13C-NMR spectrums may be shown in the text.

As the compounds are all known compounds we do not think that there is a need to report the NMR data as they can still be obtained from the literature references provided.

Associate Editor Comments

Dear authors,

The subject is interesting but I have some suggestions to improve paper's quality:

Activity part of paper was well written. But phytochemical part needs big improvement. I have serious concerns about structure elucidation of compounds. For example, you gave literature 18 for the spectral data of compound 7. But, when I read the literature, there is no spectral data of that compound. So, please supply appropriate for the all compounds throught the manuscript.

Thank you for your suggestion, reference 18 for compound 7 has been changed in the manuscript as well as reference 17 for compound 6. References related to others compounds are still appropriated.
Moreover, you done a lot of spectral experimet but not give any data. A reader should not have any doubt about structues of compounds. As, 2. reviewer suggested, you should give NMR data of some compounds, for example compound 3. Besides, you should supply which solvent was used for NMR experiments. Furthermore, you can give at least Mass value of all compounds.

- NMR data have been provided in the manuscript for compound 3.
- The MS data for all the compounds and their molecular formula have also been supplied.
- Solvents that were used for NMR experiments have been included in the manuscript.