Reviewer's report

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

Version: 1 Date: 24 May 2013

Reviewer: Sandy van Vuuren

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Date of completion of review: 20/05/2013

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.

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 overview of general medicinal plant use which is well known and frequently cited in numerous ethnopharmacological publications.

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

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.

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.

6.) Cytotoxicity

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

**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.