Author's response to reviews

Title: Antifungal and antibacterial activity and chemical composition of polar and non-polar extracts of Athrixia phylicoides determined using bioautography and HPLC

Authors:

Lyndy J McGaw (lyndy.mcgaw@up.ac.za)
Victor P Bagla (drvictorb@yahoo.com)
Paul A Steenkamp (psteenkamp@csir.co.za)
Gerda Fouche (gfouche@csir.co.za)
Jana Olivier (jana1@mweb.co.za)
Jacobus N Eloff (kobus.eloff@up.ac.za)
Martin S Myer (myer.msm@gmail.com)

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Author's response to reviews: see over
5 July 2013

The Editor
BMC Complementary and Alternative Medicine

Dear Editor

Re: Submission of revised manuscript (MS: 1093847881882996) for consideration for publication in BMC Complementary and Alternative Medicine

Herewith, I would like to submit the revised manuscript entitled “Antifungal and antibacterial activity and chemical composition of polar and non-polar extracts of *Athrixia phylicoides* determined using bioautography and HPLC” for consideration for publication in BMC Complementary and Alternative Medicine.

The reviewers are most gratefully thanked for their careful and thorough evaluations of the manuscript which led to a marked improvement of the submission once we had addressed the concerns raised. There were many valuable suggestions which have improved the paper significantly. We have attached a document detailing the responses we have made to the reviewers’ comments.

Thank you for considering this manuscript for publication.

Yours sincerely

(Dr) Lyndy McGaw
Senior Lecturer and Deputy Leader, Phytomedicine Programme
Antifungal and antibacterial activity and chemical composition of polar and non-polar extracts of *Athrixia phylicoides* determined using bioautography and HPLC

**Response to reviewer comments** *(author response typed in blue font)*

Associate Editor comments:

Page 3

Methods:
- By which procedure extracts were prepared? In my opinion, it is better to specify it at this beginning -Write compounds, but....

More information has been included on page 3 regarding the extract preparation.
A comma was inserted in the relevant position.

Page 4
L4: write anthelmintic instead of anthelmintics, cough instead of coughs

This has been changed.

Page 6
Write Materials and methods instead of Methods alone and can be ordered as:
- Plant material
- Preparation of extracts
- and son one

They cannot be combined as its written

These changes have been effected.

Page 7
HPLC: better to specify the used phases as phase A and phase B.

This has been changed as suggested.
HPLC: parag.2. L4: write (Figure 1).
L6: write Figure 2
parag.3 L2: write In figure,...
This section has been rewritten following recommendations of another reviewer.
L3: The chemical structures of these compounds belong to one and same class of phytochemical (flavonoids) and not three classes
This has been addressed and the reference to three classes of flavonoids deleted.
To write as.....noted at Rf.....: I suggest to change writing as noted to spot with Rf...... in all text because as it is written, it means that Rf in a compound or as spot and this is not clear
This suggestion has been taken into account and the manuscript modified accordingly.

Page 18
Toxicity of extract against vero cells and bring larvae: at 100 to 400 µg/ml, the extract or sample can be considered as a toxic because the concentration is very high. According to Kuyppers et al. 2006, a sample (extract) is considered as toxic if its concentration is < 32 µg/ml against a selected cell. In my opinion, the tested extracts against Vero cells is not toxic or have not a relative toxicity for the reason evoked above.
This comment has been carefully considered and the relevant sections in the manuscript revised to take this into account. The Kuypers reference refers to toxicity of isolated compounds and it may be anticipated that extracts will have a lower toxicity owing to dilution of the concentration of toxic compound(s) with other non-toxic compounds.
The only inference to be drawn from the cited reference regarding toxicity of the extracts in the manuscript is that the ethanol extract was slightly more toxic than the water extract when considered relatively, although the actual toxicity is not significant.

Figure 7: give the mobile phase
The details of the mobile phase have been included in the Figure heading.
Figure 1, 2, 5 and 6: Why there not any data on or in these figures? Not easy to understand them.
This need a revision.
The figures have been reevaluated and some deleted. Only those with the most useful information have been retained, and they have been more fully described in the text.
Editorial comments:

- Please include details in your manuscript on who identified the plant material used in your study.

The plant material was identified by taxonomists at the South African National Biodiversity Institute (SANBI), a national reference centre and herbarium. Individual scientists are not identified, but the taxonomist allocated the task of identifying the plant material will confirm the identity of the submitted voucher specimen against reference herbarium specimens.

Reviewer: Daniela Rigano

Reviewer's report:

The manuscript by McGaw et al. deals about the study of the chemical composition and antibacterial activity of Athrixia phylicoides, a medicinal plant widely used in traditional medicine of South Africa. In my opinion the manuscript is not acceptable in its present form for the publication on BMC for two main reasons. First of all, the only element of novelty in the research performed by the authors is the study of the antibacterial activity of the extracts, that has never been studied before. On the contrary, the statement that “the chemical composition of Athrixia phylicoides has also not well studied” (page 5 lines 18-19) is not true, because in literature there are different papers concerning the study of the phenolic compounds of the plant, all papers that the authors don’t cite:


4) de Beer, Dalene; Joubert, Elizabeth; Malherbe, Christiaan J.; Jacobus Brand, D. Use of countercurrent chromatography during isolation of 6-hydroxyluteolin-7-O-β-glucoside, a major antioxidant of Athrixia phylicoides Journal of Chromatography, A (2011), 1218(36), 6179-6186.

The referee is thanked for pointing out the new references which we have incorporated into the revised version of the manuscript. The novelty of the manuscript lies in the investigation of antibacterial and antifungal activity in extracts of the tea material prepared using a range of extracting solvents, and importantly in the correlation of TLC and bioautography results with HPLC traces of the same extracts. Some newly identified compounds were reported in the ethanol extract of the *A. phylicoides*.

Another important weak point of the manuscript is the overall planning of the text. Generally the English is good, but is really difficult for the readers to understand which are the effective results of the research carried out by the authors. Many parts of the manuscript are redundant (for instance page 9 lines 6-10, page 10 lines 2-12, and generally almost all the results and discussion paragraphs), and on the whole the readers have the impression to read a lab notebook rather than a discussion of a scientific paper. Also Tables 1 and 2 and the figures are not so useful for the readers. I suggest to the authors to revise completely their manuscript shortening it and trying to emphasize the results they obtained but that are really new and original on respect to what is already present in literature.

The manuscript has been extensively revised following critical evaluation, and the new contribution of the research emphasized. A large amount of the detail has been edited out of the manuscript as suggested. The number of figures has also been reduced. We have retained Tables 1 and 2 as we believe it is important for readers to know the eluting solvents and their composition to be able to repeat and expand upon the HPLC separation aspect.

**Reviewer:** Sylvia Mitchell

**Reviewer's report:**

There are no major compulsory revisions.

Minor essential revisions are placed within the word document where needed.
Some are typos but some are questions that need to be answered to give more clarity to the results. Some of the corrections etc can be considered discretionary but should be taken into consideration in making the corrections.

This paper is important as it is a study of a complex mixture, extracted as traditionally used. In this sense, the paper is important but the authors seems to be more apologising for lack of results instead of indicating why such research is necessary - these types of extracts are being used daily and are of more significance to more people than research on isolated phytochemicals.

The reviewer is thanked for her meticulous evaluation of the manuscript, providing many valid and useful comments which helped us to improve the manuscript substantially. The suggestions have been taken into account and incorporated in the redrafting of the manuscript.

In response to some of the specific comments:

A picture has been included (Figure 1).

The tea has been referred to as “Zulu tea” throughout the manuscript in preference to “bush tea” as it is true that many other teas prepared from different species are known as bush tea.

More comprehensive information on the bioactivity and toxicity of the species has been included in the Introduction.

The Figures have been evaluated and some deleted where they do not provide sufficiently valuable information.

Reviewer: Bibi Sedigheh Fazly Bazzaz

Reviewer's report:

(1) Keywords should rearrange in alphabetical orders.

This has been done.

(2) Please specify the condition of probe for sonication. (Methods, preparation of extracts)

The details and make of the sonicator have been included.

(3) Culture media’s factory must be specified. (Methods, Bacterial and fungal cultures)

These details have been included.

(4) When writing the genus and species of bacteria, fungi or plant, either in abstract or in the body of paper, for the first time must be written complete, in the next times the abbreviation of the genus
must be written. Also, if two words start by the same letter, (Candia and Criptococcus) the first syllabus must be written. Here this rule is not considered through the manuscript.

The full names of the organisms have been given the first time they are mentioned, and Candida and Cryptococcus have been typed out in full throughout the manuscript to avoid confusion.

(5) In tables or figures the comment (4) should be considered (e.g. in table 3).
The full names of the bacteria and fungi have been described in the legend of this table.

(6) In the legend of table 3, 1-4 and 5 should be explained (different extract).
The identities of the extracts have been included in the le