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

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Author's response to reviews: see over
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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 reviewer is gratefully thanked for her evaluation of the manuscript and useful suggestions which have improved the paper. We have attached a document detailing responses made to the reviewer’s comments, and all other minor corrections have been made in the manuscript.

Thank you for considering this manuscript for publication.

Yours sincerely

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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)

It is still not clear:

1) if the traditional tea is a decoction (plant material from the plant is added to water that is then boiled for a specified time) or an infusion (plant material added to boiled water and left to steep for a specific time).

Page 5 describes a traditional decoction process, but then it is referred elsewhere to an infusion.

We apologise for the lack of clarity and have addressed this issue in the paper. It becomes confusing as *Athrixia phylicoides* can be prepared as either a decoction or an infusion depending on the person using it and for what purpose it is used but this variation in preparation method has not been investigated thoroughly and reported in scientific literature at this stage. The information published on preparation for consumption as a tea refers to preparation of a decoction and this has been made clearer in the manuscript.

On page 15, both boiling (decoction) and soaking (infusion) are referred to as an infusion. Why this is important, is that boiling in hot water (for a stated period of time) and soaking in hot water (for a stated period of time), the former having heat applied during the time period and the latter having the heat removed, can affect the amount and type of phytochemicals extracted. This is important as the paper claims that the water extract is similar to that obtained traditionally.

As above, we regret the confusion and are in agreement with the reviewer’s comment that different methods of extraction with the same solvent may result in a different phytochemical profile. However, in earlier research we undertook (McGaw LJ, Steenkamp V, Eloff JN: *Evaluation of Athrixia bush tea for cytotoxicity, antioxidant activity, caffeine content and presence of pyrrolizidine alkaloids*. *J Ethnopharmacol* 2007, 110:16–22.) it was discovered that infusions, decoctions and aqueous extracts of *A. phylicoides* were very similar in terms of selected
phytochemical composition, antioxidant activity and cytotoxicity. This information has been included in the manuscript.

On page 15, the sentence has been rewritten to state that “The traditional way of preparing a herbal tea involves boiling plant material in water to yield a decoction, or soaking plant material in hot water to produce an infusion.” In this study the aim was to obtain further information on the total chemical composition of *Athrixia phylicoides* and to note the correlation of this chemical composition with antibacterial and antifungal activity, given that traditional preparations may be made in several different ways. If we have an indication of biological activity in organic extracts this may promote the idea of fermentation prior to brewing the tea to make available more of these bioactive compounds, for example.

2) In the materials and methods, it is unclear if the water extract was made with hot or cold water, as a decoction or infusion, and for what time period. Again, this is important because if the water extract is not made as tradition dictates, then one cannot compare the pattern of phytochemicals extracted and their antimicrobial activity of your extract with a traditional extract.

It has been clarified in the manuscript that the aqueous extract tested was prepared as a cold water extract following a standard laboratory extraction procedure to allow standardization, particularly as previous work had found that infusions, decoctions and laboratory-prepared cold water extracts displayed similar selected phytochemical composition, antioxidant activity and cytotoxicity as described above.

3) Some references still seem to be missing. At least the following:

* Chabeli (2006) – tannins
* Mudau 2007c
* Rakuambo ZT (2007) – Indigenous knowledge of bush tea and effect of fertigation

These references as well as others found following another careful literature survey were included in the Introduction.
All the suggestions in the table have been accepted and modifications made where necessary in the text of the manuscript, apart from that specifically mentioned below.

In response to the comment “The compounds that were identified, were these major or minor peaks on the chromatograms? And do you have Rf values for them?”

Unfortunately we do not have this information correlating the identity of the compounds to the actual peaks on the chromatograms. We have included a comment in the Discussion that further work may involve investigation into the correlation of the bioactivity of some of these compounds to their position on the HPLC chromatogram.