Author's response to reviews

Title: The in vitro antifungal activity of flavonoids against Trichophyton rubrum is due to the inhibition of fatty acid synthase and a reduction in ergosterol content

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Version: 4 Date: 1 July 2013

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

Enclosed please find the revised version of our manuscript “The in vitro antifungal activity of flavonoids against *Trichophyton rubrum* is due to the inhibition of fatty acid synthase and a reduction in ergosterol content”, which we are resubmitting for consideration for publication in BMC Complementary and Alternative Medicine.

We thank you and the referees for their valuable comments, which helped improve our manuscript. Most of the suggestions of the referees were incorporated in the text and some viewpoints are discussed. Below is a detailed list of the changes made in the text and the replies to the referees.

We hope the manuscript is now acceptable for publication and remain at your disposal for further clarification.

Sincerely yours,

Professor Ana Lucia Fachin,
Corresponding Author.

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**REPLY TO REFEREE 1:**

**Reviewer's report**

**Title:** The in vitro antifungal activity of flavonoids against *Trichophyton rubrum* is due to the inhibition of fatty acid synthase and a reduction in ergosterol content. **Version:** 3  **Date:** 11 April 2013. **Reviewer:** Joao Lago. **Reviewer's report:** No revision - this article could be accepted for publication in BMC Complementary and Alternative Medicine. **Level of interest:** An article whose findings are important to those with closely related research interests. **Quality of written English:** Acceptable. **Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Reply:** We thank the referee for the comments. With respect to the statistical review, please see the comments of referees 2 and 3.

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**REPLY TO REFEREE 2:**

**Reviewer's report**

**Title:** The in vitro antifungal activity of flavonoids against *Trichophyton rubrum* is due to the inhibition of fatty acid synthase and a reduction in ergosterol content. **Version:** 3  **Date:** 23 April 2013. **Reviewer:** Susana Zacchino. **Reviewer's report:** This revised version has been improved by authors according to the reviewers' suggestions and therefore it can be accepted for publication in BMC. However there is an issue that authors must solve. Within results, they forgot to make comments on the results of Table 2 referring to EAC, Gen, lut, and gal. A comment is also needed on why luteolin (which is active) was not included in this study. **Level of interest:** An article of limited interest. **Quality of written English:** Acceptable. **Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Reply:** We thank the referee for the comments. The results of Table 2 referring to EAC, Gen, Lut, and Gal are discussed in the new version of the manuscript.
Reviewer's report

Title: The in vitro antifungal activity of flavonoids against Trichophyton rubrum is due to the inhibition of fatty acid synthase and a reduction in ergosterol content. Version: 3 Date: 22 April 2013. Reviewer: Long N Nguyen

Reviewer's report:
The authors studied the antifungal actions of natural flavonoids such as quercetin, trans-chalcone to growth of the fungus Trichophyton rubrum by MIC and correlated the antifungal activity of these compounds to gene expression of few lipid metabolism genes in the fungus. As requested previously by this reviewer that authors should consider to test whether palmitate or oleate could reverse the effects of these compounds on the growth of Trichophyton rubrum. This is a very simple assay, but informative to test whether inhibitory effects of these compounds are on Fas1, Fas2 activity. Although, authors provided the inhibition of FAS activity in extracted proteins, this assay is insufficient to show that these compounds inhibit FAS activity. Furthermore, authors has used cerulenin for gene expression study, why it was not used as a positive control for these assays. Additionally, the MS contains many typos

Level of interest: An article of limited interest. Quality of written English: Not suitable for publication unless extensively edited. Statistical review: No, the manuscript does not need to be seen by a statistician.

Reply: We thank the referee for the comments. However, some views should be discussed. First, several papers have used the same experimental approach of the enzymatic kinetic assay to demonstrate FAS inhibition as done in the present study (see Li et al., 2002; Puig et al., 2008; Sun and Tian, 2009; Liu et al., 2009). Within this context, the enzymatic kinetics of FAS can be evaluated by measuring palmitic acid production or the consumption of intermediate metabolites based on decreasing NADPH absorbance, as done in the present study (Bays, Hill and Kariv, 2009). We disagree with the referee and consider that this assay is sufficient to suggest that our compounds inhibit FAS activity. However, we recognize that further experiments are useful to better understand the mechanism of action of these compounds. Following also the suggestion of the editor, we address the concern of the referee as limitations in the Discussion section of the revised version of our manuscript.

The assay suggested by the referee can be considered simple only if the addition of 16 carbon (palmitic acid) or 18 carbon (oleic acid) fatty acids could or not influence the action of trans-chalcone and quercetin on fungal growth. However, this would require a series of complementary experiments to confirm this hypothesis which is not in the scope of this article. Indeed, in Neurospora crassa, McKeon et al. (1997) observed a very small change in lipid composition when the fungus was cultured in medium supplemented with palmitate or oleate, what may also be the case for T. rubrum. In addition, the assay using exogenous lipids would not be conclusive to confirm that the compounds act directly on FAS, but would rather suggest that these compounds act on fatty acid homeostasis in fungal cells, as reported by Xu et al. (2012) who tested an fatty acid synthesis inhibitor in C. albicans and S. cerevisiae cells. Furthermore, if the compounds, particularly trans-chalcone, act on targets other than FAS, the effect observed in the supplementation assay with oleate or palmitate would be irrelevant. Nevertheless, we do not rule out the possibility to perform the assay in future studies since we agree with the referee that this supplementation, in combination with other tests, would contribute to a better understanding of the mechanism of action of these compounds.

Finally, the fact that we did not use cerulenin as a positive control in the FAS inhibition assay does not invalidate our results.

The manuscript was carefully revised for typographical and grammatical errors by an English-speaking person with experience in the editing of scientific text.

References:


