Author’s response to reviews

Title: In vitro antioxidant and antimalarial activities of leaves, pods and bark extracts of Acacia nilotica (L.) Del

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Dated 25-4-2017
Dear Editor,

Thank you very much for your valuable suggestions and encouraging remarks. We have revised the manuscript as suggested by the reviewers. Kindly find the response to reviewers’ comments below.

Reviewer # 1

The authors have made necessary revisions as suggested. Now it is suitable for publication. However, the quantum of work is very low. The authors failed to carry any in-vivo antimalarial activities. The work may have novelty. But does it qualify for original full length research article.

Response to reviewer # 1

Thank you for your suggestion, Acacia nilotica extracts (leaves, pods and bark) were tested in vitro for antioxidant and antimalarial effects. Thus, the manuscript depicts significant biological activities of A. nilotica extracts that makes the manuscript suitable for publication in BMC Complementary and Alternative Medicine. The future recommendation has been revised in the conclusion section of manuscript to emphasize future work needed, i.e. isolation of active compounds and in vivo characterization of antimalarial, antimicrobial and antioxidant effects. “Further purification, isolation and identification of active compounds are needed to develop novel antimalarial drugs and to characterize in vivo effects and antimalarial mechanism of action”.

Reviewer # 2

Comment 1: Your test article is not chemically characterized. In order to understand mechanism of action the chemical constituents should be identified or should be researched for this study and a scientific rationale provided as to how and which of the mixture components are contributing to the observed effects reported in your study. Phytochemical screening of the extract is important.
Response: Thank you for your suggestion. In our previous publication in the journal “Industrial Crops and Products, Volume 77, Pages 873-882 (2015)” we evaluate the chemical composition of A. nilotica extracts. These results were briefly discussed in the current manuscript, line 212-225. However, it is difficult to predict the response of individual components as it requires a substantial follow up study that we have recommended in the revised manuscript.

Comment 2: "Plant extracts were prepared by dissolving plant extracts in 40% (v/v) ethanol" is ethanol safe?

Response: Stock solutions of extracts (2 mg/ml) were prepared in 40% ethanol and serially diluted in culture medium (RPMI-1640 supplemented with 0.5% Albumax) to achieve final concentrations, ranging from 160-0.156 µg/ml in 96 wells drug plates. The plant extract showed complete mature schizont inhibition at concentrations containing negligible ethanol concentration.

Comment 3: How the authors calculated the Hill-slope?

Response: The IC50 and Hill-slope values were calculated by the non-linear regression functionality in GraphPad Prism, i.e. dose vs. normalized response model with a variable slope. The statement has been revised in the statistical analysis section of manuscript for clarification.

“Lipid peroxidation and antimalarial activity was evaluated by using the non-linear regression functionality in GraphPad Prism® version 6.01 (San Diego, US), i.e. dose vs. normalized response model with a variable slope. IC50 and Hill-slope values were computed by fitting relative activity at different concentrations of plant extracts and controls. The IC50 value was defined as concentration of tested drug or plant extract resulting in 50% of maximum effect compared to negative control (no drug). The Hill-slope was defined as the steepness of the dose-response curve”.

Comment 4: What the antimalarial mechanism of the extract?

Response: The current study focuses on in vitro evaluation of crude extracts of A. nilotica, and it is therefore, difficult to estimate the mechanism of action of crude extract. The conclusion has been revised to emphasize future work needed.

“Further purification, isolation and identification of active compounds are needed to develop novel antimalarial drugs and to characterize in vivo effects and antimalarial mechanism of action”.
Comment 5: "Tukey tests were carried out to determine significant group differences (p < 0.05)"
I did not find statistics in the work.

Response: The statistical methodology was explained in statistical analysis section, and the results from these analyses were presented in main text and also in relevant figure captions.

Reviewer # 3

This manuscript describes the evaluation of antioxidant as well as the antimalarial activities of the ethanolic extracts of three organ parts from Acacia nilotica. The authors used several well-known methods to determine the antioxidant activity. The antiplasmodial activity was determined using schizont maturation inhibition assay.

There are several previous reports on these activities of this plant species. In my opinion, the topic of the manuscript is not of a high scientific importance since the investigated plant is well-studied species and even these activities (antioxidant and antiplasmodial) were previously investigated and several reports were already published. So, the novelty is missing in this manuscript. Nevertheless, I can't neglect that a lot of work was done and the paper is well written and that pharmaceutical and pharmacological relevance is somehow still there. So, I think it can be accepted for publication after major revision. There are few points of the manuscript that need revision before acceptance. The authors are requested to consider some few points listed below.

Comment 1: Although the overall language is very good, there are some errors, e.g. format/style, grammar/syntax, and/or spelling/typographical errors. The authors would therefore be advised to read through the manuscript carefully to eliminate these typos.

Response: Thank you very much for your suggestion. The manuscript has been checked and typographical mistakes have been rectified in the revised manuscript.

Comment 2: Name of the plant species as well as bacterial or protozoal strains should be written italic in all parts of the manuscript (especially in references). "In vitro" should be also written italic (in title and many references).

Response: Edited accordingly.
Abstract:

Comment 3: Page 2/line 27: Write the full name of the plant for the first time. Plants extracts should be corrected to plant extracts.

Response: Corrected as suggested.

Comment 4: Page 2/line 30: against falciparum…. Write the full name of the parasite.

Response: Corrected as suggested

Comment 5: Give an account on the results of LC-MS and HPLC.

Response: Thank you for your suggestion, in our previous publication in the journal “Industrial Crops and Products, Volume 77, Pages 873-882 (2015)” we evaluate the chemical composition of A. nilotica extracts using LC-MS and HPLC. A brief summary of these results was incorporated in the current manuscript (line 212-225).

Introduction:


Response: Changed in the revised manuscript.

Comment 7: Last paragraph (line 68-76): needs a reference.

Response: The references have been incorporated in the revised manuscript.

Methods:

Comment 8: Line 80: Preparation of extract: Plants should be plant. This part is also too long. Reduce and rephrase.

Response: The suggested changes were incorporated in the revised manuscript.
Comment 9: Line 95: write in all subtitles: determination of ……..
Response: The suggested changes are incorporated in the manuscript.

Comment 10: Lipid peroxidation is about one page. It is too long. Reduce it
Response: The suggested changes are made in the revised manuscript.

Comment 11: Antimalarial activity: is too long (more than one page). So, reduce it.
Response: The suggested section has been reformulated in the revised manuscript.

Comment 12: Add a paragraph for the LC-MS and HPLC methods (Briefly write about the mobile and stationary phases, conditions of the experiments, etc…)
Response: The requested information is already published in our previous publication in the journal “Industrial Crops and Products, Volume 77, Pages 873-882 (2015).” We have added a brief summary of these results in the revised manuscript (see above comment).

Results and discussion:
Comment 13: In general, the discussion was poor. A good comparison and discussion with literature data was missing in certain paragraphs of this part. Compare and discuss the obtained results with published data. There are some references which were not cited such as:
Response: The discussion has been revised and the suggested information has been incorporated in the revised manuscript.

“A. nilotica is a plant rich in phenolic compounds and alkaloids that might be responsible for the antimalarial effects. A. nilotica root extracts showed a significant dose-dependent reduction in parasite densities of Plasmodium berghei in infected mice [21].”

“Methanol extracts of A. nilotica stem bark showed in vitro antimalarial effects with IC50 values of 73.59 ± 2.87 and 70.33 ± 1.89 µg/ml against chloroquine resistant and sensitive P. falciparum strains, respectively [22].”

“In a previous research report methanol extracts of A. nilotica seed and husk showed IC50 values less than 5 µg/ml against both chloroquine sensitive and resistant strains of P. falciparum [24].”

“The leaves of the A. nilotica were found to be rich in phenolic compounds compared to pods and bark extracts [14]. This might explain the higher antimalarial activity of leaves compared to pods and bark. The phenolic compounds in plant extracts have been shown previously to exhibit antimalarial effects [26], supporting the higher efficacy of A. nilotica leaves due to high phenolic content.”

Comment 14: Try to find a correlation between the antimalarial activity and the results of LC/MS.

Response: Thank you for your suggestion. Possible links between antimalarial activity and LC/MS results have been incorporated in the discussion in the revised manuscript.

“The leaves of the A. nilotica were found to be rich in phenolic compounds compared to pods and bark extracts [14]. This might explain the higher antimalarial activity of leaves compared to pods and bark. The phenolic compounds in plant extracts have been shown previously to exhibit antimalarial effects [26], supporting the higher efficacy of A. nilotica leaves due to high phenolic content.”
Comment 15: I couldn't understand the values of the compounds (chemical content) in page 12 (line 227-234). What does 87,502 mg/kg mean?? This a very very high content which is impossible. This number means that about 87 g of gallic acid is present in 1 kg of the plant!!!!!!! The standard error values are also so high. Thus, correct what is wrong or explain what you mean withes values.

Response: The values were expressed as mg/kg, resulting in 87.5 ± 0.15 g of gallic acid per kg of extract (if express as g/kg) or 87.5 ± 0.15 mg of gallic acid per g of extract. A. nilotica is a plant rich in phenolic compounds and previously it was reported that the pods of A. nilotica contained 657 mg of GAE/g of extract and when quantified by HPLC, gallic acid content was 105 ± 7.14 mg/g of extract (Singh et al., 2009). Thus, this is similar to that reported here.


Comment 16: Last paragraph in discussion: line 267: the value of artemisinin crude extract….. it should be corrected to Artemisia annua crude extract

Response: Corrected as suggested

Conclusion:

Comment 17: There is no need for the sentence in line 276: crude extract of Artemisia….. This should only be mentioned in the discussion.

Response: The suggested change has been incorporated in the revised manuscript.

References:

Comment 18: Ref. 1: the pages should be given.

Response: The complete reference has been incorporated in the revised manuscript.

Comment 19: There are few mistakes in the references Ref 4, 5, 6, 13, 16, 20, 22.

Response: The mentioned references are corrected in the revised manuscript.
Kind regards,

Anil Kumar Anal