Reviewer's report

Title: Evaluation of antimalarial activity of leaves of Acokanthera schimperi and Croton macrostachyus against Plasmodium berghei in Swiss albino mice

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Reviewer: Fabrice Boyom

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Title: Evaluation of antimalarial activity of leaves of Acokanthera schimperi and Croton macrostachyus against Plasmodium berghei in Swiss albino mice
By
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REVIEW REPORT
By Professor Fabrice Fekam Boyom

- Do not italicize “in Swiss” in the title.

Abstract
1) Results section
- Given that parasitaemia was suppressed in dose dependent manner at all dose levels, authors should delete the sentence “The highest suppression was observed at the higher dose tested (600mg/kg).”

2) Conclusion
The conclusion is not consistent, please work on this.

Background
- 1st paragraph
- Delete “in sub-Saharan Africa” in the first sentence.
- 2nd paragraph
I suggest that the following two sentences be formulated into one: “Ethiopia is also one of the most malaria epidemic-prone countries in Africa. Rates of morbidity and mortality increase 3-5 folds during epidemics [5].” For example:
“Ethiopia is also one of the most malaria epidemic-prone countries in Africa where rates of morbidity and mortality increase 3-5 folds during epidemics [5].”
- 3rd paragraph:
Today, herbal products are being used worldwide in a variety of healthcare settings, and as a home remedies [8]. What do you mean by “and as a home remedies”?
- 4th paragraph:
1) A study reported that methanol extracts of C. macrostachyus have shown substantial antimalarial [...] with IC 50 value of 0.94µgm/ml [14].

Give details about the plant parts used in the study.
- Authors should also make mention of previous studies on related plant species [ex. Croton zambesicus- Boyom et al., J.Ethnopharmacol. 123, 483-488 (2009)].

2) An in vivo study conducted “in Kenya” on Acokanthera oppositifolia, a taxonomically related species to A. schimperi, demonstrated an interesting antiplasmodial activity on P. falciparum [16]

- If you chose to provide such details, please do throughout!

- The highlighted on in [antiplasmodial activity on P. falciparum] should not be italicized.

Methods
- Plant material collection sites
  Authors should give precisions on the plant part collected (young?, old? fallen leaves?).
  The reference identification numbers of the voucher specimens at the Institute should be provided.
- Preparation of crude plant extracts
  1) The powdered plant materials were packed in plastic bags and kept in a clean and hygienic place so that no contamination occurred until extraction.

  What do you mean by hygienic place? What type of contamination could occur? I don’t think such details are necessary...

  2) The coarsely powdered plant materials were weighed using sensitive balance and repeatedly extracted in water and methanol solvents in maceration flasks.

  3) I suggest that authors give the starting plant material quantity instead of this descriptive approach; Delete solvents.

  4) Justify the 24 hours and 72 hours extraction for water and methanol.

  5) The methanol extract was concentrated at 40°C with a rotary evaporator (Buchi Rota vapor,) in distillation flask to eliminate methanol from the crude extract and concentrated further to dryness in a water bath.

  - Please delete the highlighted text; what was the temperature of the water bath?

  6) Then, the extracts were stored in tightly closed bottle containers in a freezer at 4°C…

  Did you mean a refrigerator?

Experimental animals and parasite inoculation
Experimental animals
- Provide information on mice sex and weight.
- On weekly basis, the parasites were maintained by serial passage of blood from infected mice to non-infected ones.
This should be based on parasitaemia levels. Please elucidate.

3) Parasite inoculation

Albino mice previously infected with P. berghei having variable parasitaemia.

What do you mean by variable parasitaemia?

In vivo toxicity test of the crude plant extracts

1) The crude methanol and aqueous extracts of Croton macrostachyus and Acokanthera schimperi intended for the antimalarial test against P. berghei...

Please delete the highlighted text.

2) The mice were starved 3-4hrs...

Please be consistent. Either you use hours or hrs throughout the manuscript.

3) Then, the mice in group 1 were given orally 2000 mg/kg body weight in single dose volume of 0.2 ml of the extract.

This sentence should be edited. For ex: Then, the mice in group 1 were given orally 0.2 ml of 2000 mg/kg body weight of the extract in single dose in dH2O.

4) The mice in the control groups received 0.2 ml of respective vehicle of the extract (dH2O).

Please delete respective.

5) For sub-acute toxicity studies, [...] were used for measurement.

Please add an “s” to measurement.

For this particular study, how were animal divided into groups? And how many per group?

In vivo antimalarial screening

1) I suggest that authors describe their approach in a concise stepwise way to make it easy to understand and repeat.

2) Formulas of % Suppression and % Parasitaemia should be well written.

3) On the fifth day (D4), blood samples were collected from tail snip of each mouse [23] and thin smears were prepared and stained with 10% gemisa solution.

It should be giemsa and not gemisa!!

4) Packed cell volume measurement was done before infection on day 0 and on day 4. Blood was collected from tail of each mouse in heparinized microhaematocrit capillary tubes filled up to 3/4th of their length. Then, the blood was centrifuged using microhematocrit centrifuge and measured using a hand scale reader [26].

Such a description cannot enable someone to repeat your experiment!! Please provide a concise stepwise description.

Results
Acute toxicity test
1) In the in vivo acute toxicity studies of the plant extract,….
   Add an “s” to extract.
2) Sub-acute toxicity test
   Methanol and aqueous extract of Croton macrostachyus…
   Add an “s” to extract.
3) In sub-acute toxicity studies of methanol and water extract…
   Add an “s” to extract.
4) Antimalarial activities
   Activities and not aactivities.
5) PCV and body weight measurements on day 4 indicated that both methanol and
   aqueous extract of Acokanthera schimperi…
   Add an “s” to extract.
6) page 12, 2nd paragraph:
   - The mean survival time of treatment group ranged from 7.00±1.73 up to
     10.60±0.51, …. Please add the units.
   - The mice treated with the extracts at 600mg/kg survived longer than those in
     the negative control group for both solvents (P<0.05).
     This should be highly discussed!!
Discussion
1) Page 12, last sentence: Redundant, remove!
2) Page 13: The antimalarial activities exhibited by the extracts could perhaps be
   attributable to the possible presence of active compounds.
   This statement is useless and should be removed.
3) Page 13, 2nd paragraph:
   The antiplasmodial activity has been linked to a range of several classes of the
   secondary plant metabolites including alkaloids, […] of which alkaloids have
   been the most important and have shown very interesting antiplasmodial
   activities [7].
   The highlighted antiplasmodial is redundant, remove!
   Reference [7] is not enough for this, expand!
   Croton spp. generally contain diterpenoids, triterpenoids, alkaloids, flavonoids, lignoids and
proanthocyanidins [27], which have strong antiplasmodial activity.

Please expand on this!

Page 13, 3rd paragraph:
A compound is considered active when reduction in parasitaemia is # 30% [24]. Croton macrostachyus can be considered active, its methanol extract exhibited 34.33% reduction in parasitaemia at 600mg/kg and its aqueous extract showed 30.5% and 50.53% parasitaemia reduction, at 400mg/kg and 600mg/kg, respectively.

You cannot argue on compounds and conclude on extracts. Please revise this comparison and be consistent in your approach.

Page 14, 2nd paragraph:
Your discussion should be mainly based on findings on plants of the Acokanthera and Croton genera. Search for such studies to reinforce your discussion.

Page 14, last paragraph:
Please provide further discussion on the issue of extended animal mean survival time of test samples compared to negative controls.

Page 15
The comparison of plant extracts with chloroquine potency is irrelevant and should not be a highlighted part of the discussion.

Conclusion
The authors should not overestimate their findings. They should indicate directions for further investigation of the candidate extracts.

Why has this study been limited to the suppressive effect of plant extracts?

References

Tables
1) Authors should add the unit for body weight in tables 1 and 2.
2) The data presented in the 6 tables can fit into two, joining data for body weight and PCV in one, and suppressive effect of extracts in the another.

CONCLUDING REMARKS
THIS IS A GOOD CONTRIBUTION IN THE FIELD OF INVESTIGATION OF THE AFRICAN BIODIVERSITY TO SEARCH FOR ALTERNATIVE THERAPIES TO FIGHT MALARIA.

THE AUTHORS HAVE USED APPROPRIATE TECHNIQUES TO ACHIEVE RESULTS THAT SHOW MODERATE POTENCY OF EXTRACTS FROM TWO
PLANTS THAT ARE USED IN ETHNOMEDICINE IN ETHIOPIA. THE LANGUAGE LEVEL IS GOOD. IF THEY AGREE TO REVISE EXTENSIVELY THEIR MANUSCRIPT FOLLOWING THE RECOMMENDATIONS, THEREFORE IT CAN BE CONSIDERED FOR PUBLICATION IN BMC COMPLEMENTARY AND ALTERNATIVE MEDICINES.

**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.

**Declaration of competing interests:**

I declare that I have no competing interests