Author's response to reviews

Title: Antileptosporal activity of xanthones from Garcinia magostana and synergy of gamma-mangostin with penicillin G

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Version: 3 Date: 7 January 2013

Author's response to reviews: see over
4 January 2013

The Editor,

Dear Sir,

On behalf of the other co-authors, I am submitting a revised manuscript entitled “Antileptospiral activity of xanthes from *Garcinia magostana* and synergy of γ-mangostin with penicillin G” to publish in *BMC Complementary and Alternative Medicine* as an research article.

I would like to thank the editor and reviewers for valuable comments.

The manuscript was revised in the red letter correspond to the comments and suggestion from editor and the two reviewers.

A point-by-point response to the comments are shown below.

I hope that the revised manuscript is now suitable to publish in *BMC Complementary and Alternative Medicine*.

Sincerely yours,

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Response to editorial comment

- We recommend that you ask a native English speaking colleague to help you copyedit the paper. If this is not possible, you may need to use a professional language editing service. For authors who wish to have the language in their manuscript edited by a native-English speaker with scientific expertise, BioMed Central recommends Edanz (www.edanzediting.com/bmc1).

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Response: We asked for an assistant from our faculty staff who is native English speaking to edit the manuscript.
Response to reviewer’s comment

Reviewer: Lahcen HASSANI

Major points:

1- There are no keywords.

**Response:** Thank you very much for your kindly comment. The keywords are added in the manuscript as follows;

**Keywords:** *Leptospira; Mangosteen; Xanthones; gamma-Mangostin; Synergy; Penicillin G.*

2- Since the two sections “Results” and “Discussion” are not very large, I suggest they are combined into a single one “Results and discussion”.

**Response:** We combined the “Results” and “Discussion” to be Results and Discussion as your suggestion.

3- In the section “Discussion”, while comparing their results with previously published ones, the authors have to acknowledge them by citing all the corresponding references.

**Response:** The cited journals have been added in references as comment.

4- Some thoughts about the possible role of mangostin in the synergy with penicillin G should be presented.

**Response:** We added the possibility of synergy as follow;

“gamma-mangostin may work synergy with penicillin G on breakdown of bacterial membrane”

5- Lines 212 and 213: the authors proposed: “For prevention of Leptospiral infection through skin, #-mangostin or crude extract may be used as a constituent of some products such as soap, or spray to kill the bacteria”. This is not necessary true; the mixture of #-mangostin or crude extract with soap or spray could generate an antagonistic effect.

**Response:** We deleted this sentence.
6- Because the xanthone solutions used are not pure, it is possible that the synergistic and/or the antagonistic effects are rather due to impurities, not to xanthone.

Response: The prepared xanthone was 98% purity which purify enough to state that the synergistic and/or the antagonistic effects come from xanthone.

7- Please insert a section “Conclusions” which should clearly and concisely state the main conclusions of the research and give a brief explanation of their relevance.

Response: We have inserted a section of “Conclusions” in the manuscript as shown below.

Conclusions: The Garcinone C and γ-mangostin from fruit of G. mangostana active against pathogenic leptospires but the MIC values were higher than antibiotics. The combination of γ-mangostin with penicillin G generated synergistic effect which enhances efficacy for treatment of leptospirosis.

8- English and syntax are unsatisfactory; they need correction.

Response: English was edited by native English speaking staff.
Response to reviewer’s comment

Reviewer: Satendra Singh

The article entitled “Antileptospiral activity of xanthones from Garcinia mangostana and synergy of gamma-mangostin with penicillin G” showed the synergistic effect of traditional medicines with commonly available antibiotics in the market. It is a good initiation in the area of traditional medicine to combat deadly human disease and their synergistic interaction with available drugs.

But at this level, it is very difficult to accept the article for publication in the journal of CAM and work is too small to be considered for publication in the CAM. I found a lot of errors in the preparations of manuscript, which cannot be avoided. Lots of language corrections are required in whole article.

Response: Thank you for your comments. Our manuscript has been revised as your comments. However, I would like to clarify about the level of our work that you have mention. I think that the level of our work is enough to publish in CAM when comparing with other articles that have published in CAM or the similar works that had been published in other Journal with higher impact factor. Most of them contain the results of inhibition assay using commercial drugs and synergistic study. Another points are as follows,

1. In our case, we purified and identified the xanthones by ourselves because our country grow a lot of mangosteen. The purity of the xanthones were about 97- 98%. These experiments were hard work to get enough amount xanthones for experiments.

2. It is the first report that informed about inhibitory activity of xanthones from mangosteen against Leptospira spp. Even the MICs were not so low when comparing to antibiotics but it was the nature of these compounds. Nowadays, mangosteen products such as mangosteen juice, cosmetic and pharmaceutical products are popular. This data is useful for consumer, and/or industries for development new products concerning to protection of leptospirosis.

3. We also test the MIC of antibiotic, the MIC of penicillin G was in the range 0.39-3.13 mg/L (mean = 1.46 mg/L) which similar to the previous reports (MIC90=1.56 mg/L) confirmed about the standard of our experiment.

4. It is the first report concerning synergistic effect between antibiotic and xanthones from mangosteen.

5. The problem that lead to the limit of our results were follows,
5.1 Experiments that due with *Leptospira* spp. were hard work because they grow slowly, sensitive to diluent (DMSO). We started experiment with 15 serovar of *Leptospira* spp., but we lost 10 and 5 serovars were left for testing inhibitory and synergy assays.

5.2 After testing the MIC, the leptospiral cell can not further investigate for minimum bactericidal concentration (MBC) due to the contamination during the step of adding alamarBlue.

5.3 For testing the mechanism need time and budget. We got a little financial support, so we can not carry on this experiment.

6. About grammatical error, we ask for an assistant from the native English speaking of our faculty staff to edit our manuscript again.

**My observations**

1. The plant name is *Garcinia mangostana*, but it is itself in the title and many places in text have been spelled wrongly as

   **Response:** The title and other places were corrected to *Garcinia magostana*

**Abstract:**

2. Bacterial name is *Leptospira biflexa* but it is also wrongly spelled as *Leptospira Bifexa*

   **Response:** The bacterial name was corrected to *Leptospira biflexa*

3. Second sentence in second paragraph is senseless; nobody can understand what actually authors want to say. Authors are doing only antibacterial activity, use of antimicrobial word in abstract is appropriate

   **Response:** The second sentence was rewrite to “the xanthones possessed antileptospiral activities, with the minimal inhibitory concentration (MIC) varying from 100 to ≥800 mg/L”

**Background:**

**First para**

4. Authors gave many facts in the first paragraph but they mentioned a single reference. Is it sufficient?

   **Response:** We have added more references.

5. I cross checked the given reference and did not found some of the facts in this
paragraph. Exhausted literature is missing in the entire manuscript.

Response: We have added more references.

Second para
6. There is no correlation between first and second paragraph, authors directly start talking about xanthones.
Response: We have revised the first and second paragraph to be more correlated.

7. The sentence---mangostins isolated from the fruit hull of *G. mangostana*…… should be like that------- mangostins isolated from the fruit wall of *G. mangostana*.
Response: We edited as your suggestion.

8. In some place authors mentioned as anti-inflammation, anti-cancer, anti-malaria but in next line antiviral, antifungal and antibacterial. Why?
Response: We edited the repeated part.

9. Last sentences is too long and don’t have any sense.
Response: We rewrite it to be shorter.

Forth para
1. Is it conclusion? or basic theme for work to be carried? Too long and inappropriate sentence
Response: We have edited it.

Materials and Methods
First para
1. First sentence should be….the bacterial culture was obtained from the department of Medical Sciences. Word originate do not have any sense.
Response: We have edited it.

2. Last sentence……..inoculums were; vetriplast not vetripplast, entire sentence is inappropriate.
Response: We have edited it.
Second para
1. It is very difficult to understand the preparation of all crude extracts.
   **Response:** We have rewrite the preparation of crude and purified xanthones.

2. Format of citation in text is not uniform. Somewhere it is like [3] while at other places it is represented as superscript (like2).
   **Response:** The [3] represent the code of garcinone C, not the reference. We have deleted all of these number to avoid confusing.

3. Authors should re-write whole paragraph clearly.
   **Response:** We have rewrite the whole paragraph as suggestion.

Third para
1. Positive control, negative control, the plates were tightly rapped or wrapped?
   **Response:** We have edited it to wrapped

2. This is completely confusing and inappropriate sentence.
   **Response:** We have edited it.

3. Alamarblue 10 time concentrated. What does it means?
   **Response:** We have edited it to alamarBlue (10×).

Fourth para
1. Last four line…… can any reader is able to repeat this exercise? How many commas? Where to stop? It is very difficult to read and understand.
   **Response:** We have edited it.

Fifth Para
1. The name gamma mangosteen should be corrected as gamma mangostin.
   **Response:** We have edited to be gamma mangostin.

2. Why authors are repeating again and again positive and negative control? They are understood.
   **Response:** We have edited it by deleting the repeating sentence.
Results
1. Authors did not mention the MIC in results (third line). In one sentence authors are saying lower mic but they are using however mangostin was selected for testing. Total confusion in whole sentence, it seems that authors are not sure about their own results.

Response: We have edited it to be “The $\gamma$-mangostin was used in this study even it showed higher MIC than Garcinone C due to its high abundance and low MIC (100 to $\geq$800 mg/mL”).

2. Results are not sufficient to publish in journal of CAM.

Response: This manuscript contains at least 2 new data

(1) The first report about the MIC of xanthones from mangosteen against *Leptospira* spp. This result give useful information for people to use xanthones from mangosteen for treatment of leptospirosis. They can use as a supplement in food or drink for human and animal meals. In addition, it have been reported that $\gamma$-mangostin can protect human colon cancer and have antagonistic effects. These information suggest that consuming of xanthones from mangosteen can protect several diseases.

(2) The first report about synergistic effect between antibiotic and xanthones from mangosteen.

These results are comparable to other articles published during the year 2012 in Journal with high impact factor.

Discussion
1. Plant name—again spelled wrong

Response: We have edited it.

2. Antibiotic name is incorrectly written as doxycycline. It should be doxycycline

Response: We have edited it.

3. Sentence---but the role of penicillin is inhibition……………..It not selfsufficient and informative. Authors should give proper literature to support the facts.

Response: We have edited it.

4. What authors used in experiment…………….epicarp or pericarp.

Response: We have edited it to be pericarp.
5. It is not clear which serovars belongs to *L. biflexa* and which belongs to *L. interrogans*?

**Response:** Patoc is belong to *L. biflexa* but serovar Autumnalis, Bataviae, Javanica, and Saigon are belong to *L. interrogans*. 