Cover Letter for the submission of revised manuscript

(ID 3436940)

Dear Editors of

International Journal of Food Science

Thank you for the opportunity to revise our manuscript (ID 3436940) titled Antioxidant Activity, Enzymes Inhibition Potentials, and Phytochemical Profiling of *Premna serratifolia* L Leaf Extracts.

We thank both reviewers for their valuable time in carefully reading the manuscript. We have given our effort to improve our manuscript in light of the Reviewers' suggestions and comments. We hope the revised manuscript has met the journal's standard for publication. Item-wise answers to their specific suggestions/comments are as follows.

Reviewer 1

Summary: Authors have reported characterization, antioxidant and enzymatic studies of ethanolic and water extract of P. serratifolia leaves.

Reviewer's Comments	Author's Response
Abstract: further investigation is required to find the best knowledge for use of P. serratifolia leaves" insert word "medicinal" use	It has been added to the abstract (line 12).
Methodology Well written and references are quoted	Thank you
2.2 Plant Material and Extracts Preparation Why did authors select ethanolic and water extract? Why not methanolic or other fraction, include in your discussion	As mentioned in line 248 – 249, plant secondary metabolites such as those of phenolic and flavonoid derivatives have been known to be associated with various bioactivities. Polar to semi solvents (such as water, methanol, ethanol, ethyl acetate) are considered appropriate to extract these metabolites, considering heterogenous moieties of the phenolic and flavonoid derivatives. More specifically, water extraction was used since it is a common way in which it can be prepared in domestic context. It was mentioned in the introduction that traditionally *P. serratifolia* leaves were used for various medicinal benefits (line 54 – 56), which was most likely prepared by decoction or infusion. Thus, our aim is twofold, to provide

	scientific support for its ethnopharmacological uses, while potentially providing relevant application in domestic context. Regarding extraction using organic solvents, it was expected that less polar bioactive constituents in the leaves can be extracted using these solvents. Ethanol was used in the study since it is considered relatively safer compared to some other organic solvents such as methanol, CHCl3 and ethyl acetate. We have added required information in order to support the selection of ethanol and water as extractants, please see line 250-2 and line 260-2, respectively.
2.5.5 DNA protection assay Your loading volume is 17 µl and gel running time is 60 mins /60 V, some standard protocol mention 20 µl loading volume for 60 mins /90 V. Did author well validated the protocol for before running the samples? if then mention in your protocol about validation.	Thank you for your careful observations. Our apology for we are required to correct some details in the method: 1. The running time was 90 mins/80 V (correction in line 159), as at this condition we observed our results. 2. the brand of our instrument (line 160). As for the loading volume, we used 17 µL. We were careful to always have control for each change made, to confirm the results.
Results & Discussion: 1. Figures are not clear; enhance the quality to convert into 300 DPI or appropriate	We have enhanced the quality of each figure into 450 DPI.
2. LC-QTOF-MS/MS analysis of the ethanol extract: in table include class of compound identified. Authors should discuss and correlate compounds identified in other parts as well.	Thank you for your suggestion. We have included the identified compounds in the discussion of bioactivities, such as in the discussion on TP and TFC (line 255-60), antioxidant activity (line 298-300), DNA protective effect (line 337-40). We have done this for enzyme activity, such as in line 367 – 371 for α-glucosidase inhibition activity.
3. TP , TF and Antioxidant activity should be discussed with reference to your compound identified rather than pathophysiology, including pathophysiology is a good context but it does not give any innovation	Thank you for this valuable input. We have added discussion about phenylethanoid glycoside (PhGs) derivatives which were identified in the LC-QTOF-MS/MS, in the discussion of TP, TF, and antioxidant. Please see above (in point 2).
4. Your data shows that DPPH scavenging activity of your ethanolic sample has better activity than standard ascorbic acid and other assays show lower antioxidant activity. Due have any reason or explanation for this.	As mentioned in line 264-8 p7, several methods were used to assess antioxidant activity of the samples due to various mechanisms of antioxidant actions. Firstly, the antioxidant activity of the samples was

	assessed based on their ability to scavenge DPPH free radicals by way of donating protons (hydrogen atom transfer) from the antioxidant compounds in the extracts. Activities were expressed as IC_{50} values which were compared with that of the standard (ascorbic acid). The ethanolic sample shows higher activity compared to ascorbic acid. Secondly, the antioxidant activity of the extracts was evaluated based on its reducing capacity (electron donating ability/single electron transfer). In the present study, several methods were employed which based on the reduction of metal ions of higher oxidation state into their lower oxidation numbers (reduced states), i.e Cu(II) to Cu(I), Mo(VI) to Mo(V), and Fe(III) to Fe(II), for CuPRAC, phosphomolybdenum, and ferric thiocyanate methods. Activities were expressed as standard equivalents, such as Trolox equivalents and ascorbic acid equivalents. Thus, reducing activities were not compared with a positive control. However, it was observed that for all methods, ethanol extract was consistently stronger than water extract. Both types of antioxidant mechanisms (radical scavenging and reducing activities) used different expressions of results, thus not directly comparable. To enhance clarity, we have added information regarding the abbreviations used for standard equivalent used (footnote in Table 2, p7).
5.: Include both references in your discussion - Premna serratifolia anti-gout activity of wood. a. "Abu Bakar FI, Abu Bakar MF, Rahmat A, Abdullah N, Sabran SF, Endrini S. Anti-gout Potential of Malaysian Medicinal Plants. Front Pharmacol. 2018;9:261. Published 2018 Mar 23. doi:10.3389/fphar.2018.00261" b. Rajendran R., Krishnakumar E. (2010). Anti-arthritic activity of Premna serratifolia Linn., wood against adjuvant induced arthritis. Avicenna J. Med. Biotechnol. 2, 101–106	We thank Reviewer 1 for the suggested references. We have included these in our discussion, please see line 388-9.

Reviewer 2

Reviewer's Comments	Author's Response
Regarding the novelty of this study Unfortunately, there are reports on the phytochemicals, antioxidants, antidiabetic and other properties of the plant in the literature. Just to name a few, 1. see DOI: 10.5530/pj.2018.6.189, 2. DOI: 10.4103/0257-7941.179864; 3. PMID: 23284207, 4. doi.org/10.1002/ptr.5229. These undermine the novelty of this manuscript.	Thank you for your careful observations and suggested references. The first article is our previous study on *P. serratifolia*. We have mentioned this in our manuscript (currently ref no 14). The current study is indeed an extension of the previous study in which more enzyme inhibition activities (inhibition on xanthine oxidase and protease) and antioxidant activities (reducing power and DNA protective effect) were evaluated and compared with those of ethanol extract. It should be noted however that the present manuscript does not contain any similar number results with the previous publication. In the previous publications, activities were presented as IC50 with µgGAE/mL unit, designating to gallic acid equivalent. To better represent the previous work, we have added this information in the introduction (line 63-4). 2. Literature 2-4 have been added to the introduction to provide better background of the current study (line 61-3) We believe that new findings in this study such as xanthine oxidase and protease inhibitions and phytochemical profiles of the leaf extract may contribute to the current literature on *P serratifolia*.
The results from this study were not compared with other reports on the leaf extracts of the plant or the plant in general.	We have done comparisons with previous studies when discussing our results, as can be found in many parts of the manuscript: However, we have added more comparisons in light of this suggestion. Line 252-4, 280-1, 290-2, 361-2, 388-9.
The authors also concluded that "further studies are required to evaluate its toxicity" I will suggest that the authors check DOI: 10.1002/ptr.5229. It is evident that proper literature review was not done before the commencement of this study.	Thank you for your input. We have amended this (line 28 has been deleted).
The positive aspect of this study is the identified compounds. However, these compounds need to be confirmed and	We agree with Reviewer 2 for the suggestions. However, we are sorry we cannot do this in the current pandemic condition.

characterized using other methods. I will suggest that the authors focus on isolation of new compounds from the plant and determine possible bioactivities of those compounds. As it is only LCMS is not sufficient to ascertain the compounds identified in this study.	
line 33. Abnormal high enzyme activities? It would be better to explain this statement. e.g what are the enzymes that are abnormally high in diabetes?	Thank you for the input. The required information has been added, please see line 33-4.
line 34. Again, how is inflammation treatment used? give references	We have added the required information and reference (line 35-6).
line 57. The leaves have also been investigated see DOI: 10.5530/pj.2018.6.189	We concur. We have presented this in the introduction (line 63-4) and in the discussion accordingly (line 280-1 and 361-2).
The introduction needs to be rewritten	We thank Reviewer 2 for the input and have added information in the introduction accordingly.
line 78. 3 days is unnecessary for maceration. 24 hrs should be enough	Extant literature provides numerous variations on maceration, including time required. The length of maceration time should not be a significant issue, as has been reflected by findings presented in our manuscript.

This manuscript describes Antioxidant Activity, Enzymes Inhibition Potentials, and Phytochemical Profiling of *Premna serratifolia* (syn *Premna integrifolia*, *Premna obtusifolia*) Leaf Extracts using different *in vitro* approaches. Unfortunately, there are reports on the phytochemicals, antioxidants, antidiabetic and other properties of the plant in the literature. Just to name a few, see DOI: 10.5530/pj.2018.6.189, DOI: 10.4103/0257-7941.179864; PMID: 23284207, doi.org/10.1002/ptr.5229. These undermine the novelty of this manuscript. The results from this study were not compared with other reports on the leaf extracts of the plant or the plant in general. The authors also concluded that "further studies are required to evaluate its toxicity" I will suggest that the authors check DOI: 10.1002/ptr.5229. It is evident that proper literature review was not done before the commencement of this study. The positive aspect of this study is the identified compounds. However, these compounds need to be confirmed and characterized using other methods. I will suggest that the authors focus on isolation of new compounds from the plant and determine possible bioactivities of those compounds. As it is only LCMS is not sufficient to ascertain the compounds identified in this study.

These are minor revisions required to improve manuscript.

1. line 33. Abnormal high enzyme activities? It would be better to explain this statement. e.g what are the enzymes that are abnormally high in diabetes?
2. line 34. Again, how is inflammation treatment used? give references
3. line 57. The leaves have also been investigated see DOI: 10.5530/pj.2018.6.189
4. The introduction needs to be rewritten
5. line 78. 3 days is unnecessary for maceration. 24 hrs should be enough

Conclusion

I will suggest that the authors check for grammatical errors throughout the manuscript.

Summary:

Authors have reported characterization, antioxidant and enzymatic studies of ethanolic and water extract of *P. serratifolia* leaves.

Reviewers Comment

Abstract

1. "further investigation is required to find the best knowledge for use of *P. serratifolia* leaves" insert word "medicinal" use.

Methodology:

Well written and references are quoted

2.2 : Why did authors select ethanolic and water extract? Why not methanolic or other fraction, include in your discussion.

2.5.5 DNA protection assay:

Your loading volume is 17 µl and gel running time is 60 mins /60 V, some standard protocol mention 20 µl loading volume for 60 mins /90 V. Did author well validated the protocol for before running the samples?, if then mention in your protocol about validation .

Results & Discussion:

1. Figures are not clear; enhance the quality to convert into 300 DPI or appropriate.

2. LC-QTOF-MS/MS analysis of the ethanol extract: in table include class of compound identified. Authors should discuss and correlate compounds identified in other parts as well.

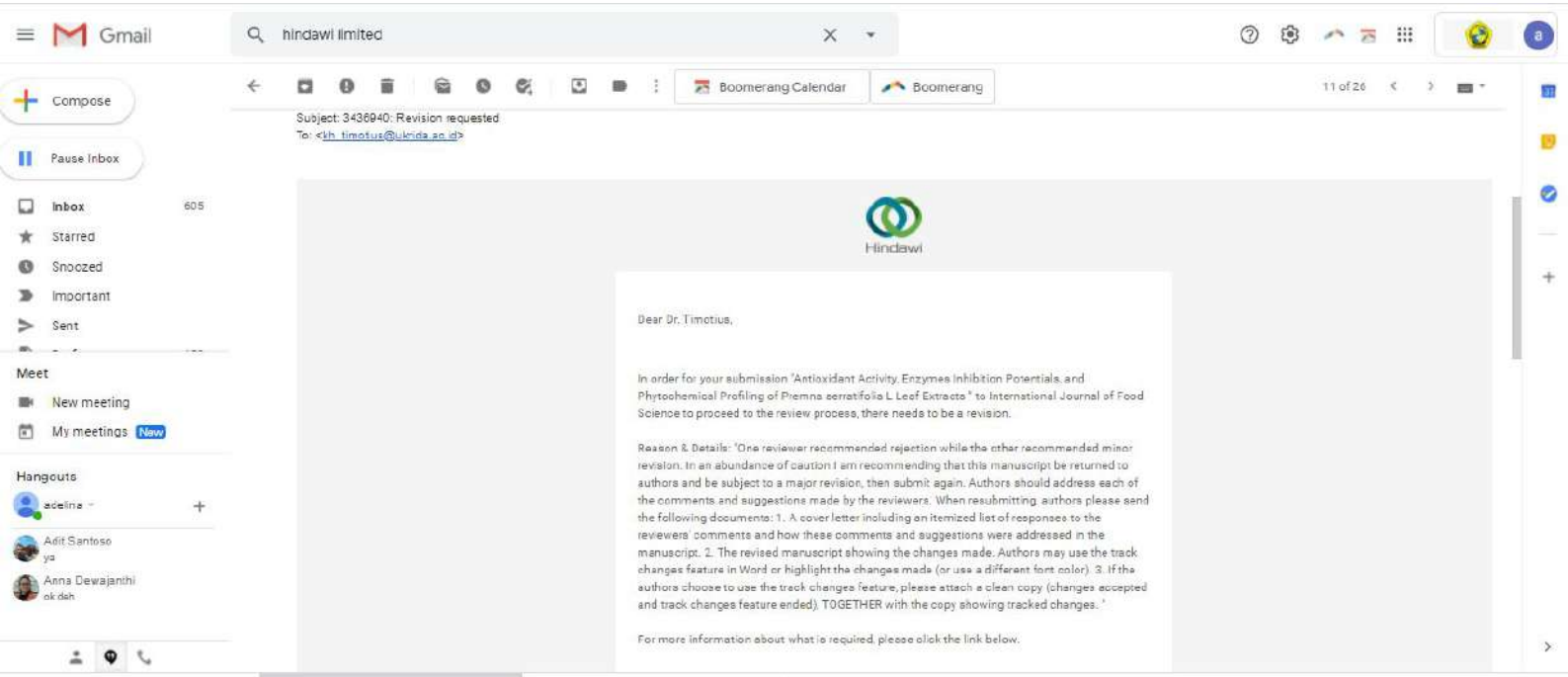
3. TP , TF and Antioxidant activity should be discussed with reference to your compound identified rather than pathophysiology, including pathophysiology is a good context but it does not give any innovation.

4. Your data shows that DPPH scavenging activity of your ethanolic sample has better activity than standard ascorbic acid and other assays show lower antioxidant activity. Due have any reason or explanation for this.

5. Anti-oxidase activity: Include both references in your discussion - *Premna serratifolia* anti-gout activity of wood.

a. "Abu Bakar FI, Abu Bakar MF, Rahmat A, Abdullah N, Sabran SF, Endrini S. Anti-gout Potential of Malaysian Medicinal Plants. Front Pharmacol. 2018;9:261. Published 2018 Mar 23. doi:10.3389/fphar.2018.00261"

b. Rajendran R., Krishnakumar E. (2010). Anti-arthritic activity of Premna serratifolia Linn., wood against adjuvant induced arthritis. Avicenna J. Med. Biotechnol. 2, 101–106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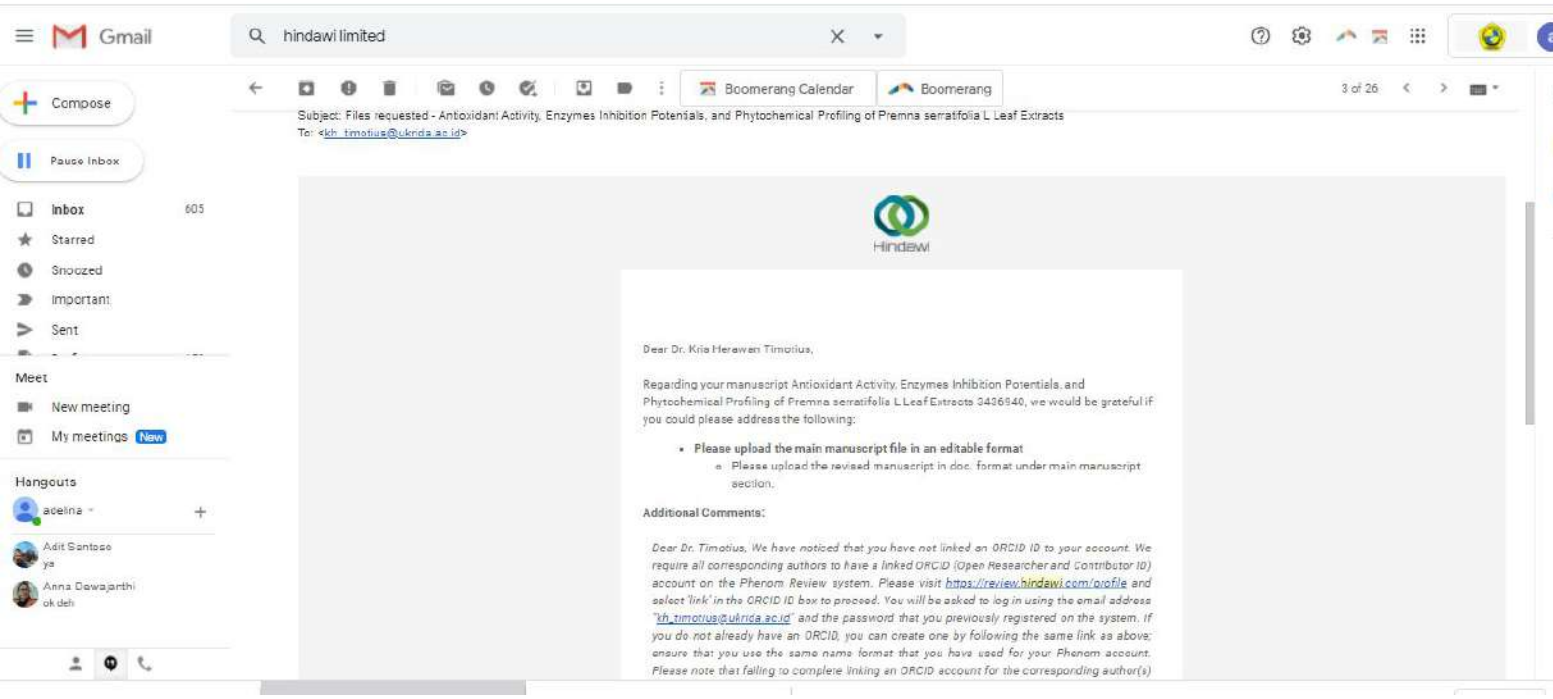
Boomerang
11 of 26
Subject: 3436940: Revision requested
To: <kh_timotius@ukrida.ac.id>
Hindawi
Dear Dr. Timotius,
In order for your submission "Antioxidant Activity, Enzymes Inhibition Potentials, and Phytochemical Profiling of Premna serratifolia L Leaf Extracts" to International Journal of Food Science to proceed to the review process, there needs to be a revision.
Reason & Details: "One reviewer recommended rejection while the other recommended minor revision. In an abundance of caution I am recommending that this manuscript be returned to authors and be subject to a major revision, then submit again. Authors should address each of the comments and suggestions made by the reviewers. When resubmitting, authors please send the following documents: 1. A cover letter including an itemized list of responses to the reviewers' comments and how these comments and suggestions were addressed in the manuscript. 2. The revised manuscript showing the changes made. Authors may use the track changes feature in Word or highlight the changes made (or use a different font color). 3. If the authors choose to use the track changes feature, please attach a clean copy (changes accepted and track changes feature ended), TOGETHER with the copy showing tracked changes."
For more information about what is required, please click the link below.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3 of 26
Subject: Files requested - Antioxidant Activity, Enzymes Inhibition Potentials, and Phytochemical Profiling of Premna serratifolia L Leaf Extracts
To: <kh_timotius@ukrida.ac.id>
Hindawi
Dear Dr. Kris Herawan Timotius,
Regarding your manuscript Antioxidant Activity, Enzymes Inhibition Potentials, and Phytochemical Profiling of Premna serratifolia L Leaf Extracts 3426940, we would be grateful if you could please address the following:
Please upload the main manuscript file in an editable format
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Additional Comments:
Dear Dr. Timotius, We have noticed that you have not linked an ORCID ID to your account. We require all corresponding authors to have a linked ORCID (Open Researcher and Contributor ID) account on the Phenom Review system. Please visit https://review.hindawi.com/profile and select 'link' in the ORCID ID box to proceed. You will be asked to log in using the email address "kh_timotius@ukrida.ac.id" and the password that you previously registered on the system. If you do not already have an ORCID, you can create one by following the same link as above; ensure that you use the same name format that you have used for your Phenom account. Please note that failing to complete linking an ORCID account for the corresponding author(s)