Dear Editors,

Thank you for the opportunity to revise our manuscript titled "In Vitro Antidiabetic and Antioxidant Activities of Aqueous Extract from the Leave and Fruit of Psidium guajava L". We would like to thank the reviewer for careful and thorough reading of this manuscript and for constructive suggestions, which we believe has helped improve the quality of this manuscript.

Following this letter are files which include the revised manuscript and file containing our point-to-point responses to the reviewer's comments. Changes made in the revised manuscript are marked in blue highlight. The revision was developed in consultation with all co-authors and each author has given approval to the final form of this revision.

Response to Revisions Request

M201743

CommentInaBJ1:

Please state what kind of drug or positive control you used for your both for α-glucosidase inhibitory and antioxidants activities research

Response:

Thank you for the suggestion. Throughout the abstract, changes have been made to include compounds used as positive controls or references for each test (line 11, 15, 16, 17, and 19).

CommentInaBJ3:

Why did you mention that LE, FE and acarbose inhibition activity were competitive? From the Result, three samples have significant difference in α-glucosidase inhibition activity.

Response:

We appreciate the feedback from the reviewer. However, the term 'competitive' refers to the enzyme inhibition mechanism. Modes of enzyme inhibition can be classified as competitive, uncompetitive, and non competitive (Mosier & Ladisch, 2009). The term was not intended to indicate the comparison among inhibition activities of the extracts and standard. The sentence in text has been re-written in order to better state this result (line 12-13).

CommentInaBJ5:

Please replace methodologies with methods.

Response:

It has been amended throughout the manuscript.

CommentInaBJ7:

Please mention clearly the value for each antioxidant activity.

Response:

Antioxidant activities have been added to to the text (line 15, 17, 19, 20).

CommentInaBJ9:

The IC50 of LE, FE, acarbose were 5.67, 428.00 and 823.99 µg/ml. These results did not show excellent inhibitory activities. Please check and compare to the previous published Journal, How to classify active, moderate, and inactive value of glucosidase inhibition activity? Please check the Published Journal, how was the IC50 of α-glucosidase inhibitory activity? Why does the IC50 of acarbose was very high? Please compare to many Published Journal. Was this method correct?

Response:

We appreciate the feedback from the reviewer. Plant extracts were concluded as having potent α-glucosidase inhibitory activities (IC50 values) when compared with acarbose (a positive control). To the best of our knowledge, no report has been published yet regarding classification of activities values (active, moderate, and inactive). However, we have re-written the statement in the abstract conclusion (line 14) in order to describe the result better. As it appears now, only LE was described as an excellent α-glucosidase inhibitor, as evidenced by its IC50 of 5.67 µg/ml (line 21). Regarding comparison of acarbose activities to other published works, please see our response below.

CommentInaBJ9:

Acarbose acts as α-glucosidase inhibitors but why did this results has a very low activity? Acarbose was the lowest activity compared to LE and FE? Please check carefully.

Response:

We appreciate the feedback. Acarbose is commonly employed as a reference standard for in vitro α-glucosidase inhibition studies. In the current study, we used acarbose produced by USP (United States Pharmacopeia) obtaining IC50 of 823.99 ± 0.06 µg/ml (3 replicates). The IC50 was obtained based on spectrophotometry method. Many literatures, however, have reported diverse values of acarbose activity (IC50). For instance: 3.45 µg/ml (Ali et al., 2013), 12.6 µg/ml (David et al., 2017), 117.06 µg/ml (Mun'im et al., 2013), 1.3 mg/ml or 1300 µg/ml (Shukla et al., 2016), 5 mg/ml (Kim et al., 2000), and 6.2 mg/ml (Subramanian et al., 2008). It is worth noting that the said values were the results of similar spectrophotometry methods with slight modifications among them. The discrepancies could probably be explained from different sources of acarbose.

In addition, many literatures have reported that various plant extracts exhibited potent inhibitory activity against α-glucosidase with activities stronger than acarbose, such as (Mun'im et al., 2013), (Yilmazer-Musa et al., 2012), and (Surya et al., 2014).

CommentInaBJ11:

Please add the correlation between antioxidant and α-glucosidase inhibitory activity toward antidiabetic potency, furthermore you investigated both activities

Response:

Thank you. A paragraph has been added to address the above suggestion (line 42-47).

CommentInaBJ13:

Please add the P. guava compounds which have antioxidant and α-glucosidase inhibitory activity, furthermore you investigate P. guava extract as antioxidant and α-glucosidase inhibitory activity

Response:

Thank you. The introduction has included this information (line 58-61).

CommentInaBJ15:

Please mention the reference you cited this from.

Response:

Thank you. The required citation has been added (line 56).

CommentInaBJ17:

Please state the village, region not only province

Response:

We have added this information in the text (line 69-70).

CommentInaBJ19:

Please presence the laboratory name (ex: Name laboratory, faculty, school)

Response:

The required information has been added in the text (line 71-72).

CommentInaBJ21:

What kind of Guava fruit did you use, Was it ripe or raw fruit. How did you dry the Guava fruit in a big size. Did you dry directly the whole fruit or chopped fruit?

Response:

We have added the Information in the text (line 73).

CommentInaBJ22:

Please mention the reference you cited this from.

Response:

Citation has been added in the text (line 75).

CommentInaBJ23:

What concentrations did you use?

Response:

The concentrations have been added (line 83-84).

CommentInaBJ24 – 47:

Please complete with catalog number.

Response:

Thank you. Throughout the revised manuscript, catalogue number for each chemical have been added.

CommentInaBJ27:

Please mention the concentration level of Gallic Acid you used. Please state the linear equation in the result section, also please complete with catalog number.

Response:

As suggested by the reviewer, concentration levels (line 90) and linear equation of the gallic acid have been added in the text (section result, line 192-193).

CommentInaBJ31:

Please state the linear equation in the result section, and also please complete with catalog number.

Response:

Correlation equation and correlation coefficient have been included in the text (section result, line 194-195).

CommentInaBJ33:

What concentration level did you use?

Response:

The concentration ranges for both LE and FE have been added in the text (line 109). The exact concentrations can be seen in Table 2.

CommentInaBJ37:

What were the exact concentrations did you use?

Response:

The concentration ranges for both LE and FE have been added in the text (line 134-135).

CommentInaBJ42:

Better if you write the exact concentration.

Response:

The concentration ranges for both LE and FE have been added in the text (line 148).

CommentInaBJ48:

Please also add information regarding the ethical approval, which consist of the ethical approval number, and the institute that gave the approval.

Response:

The current study did not use human or animal as experimental objects, therefore ethical approval was not required.

CommentInaBJ50:

This should be written in the Discussion section. In the Result section, you describe the results clearly.

Response:

This has been amended as suggested (line 196-199).

CommentInaBJ52:

Please also mention the results of the IC50.

Response:

Throughout the result section, values of IC50 have been added.

CommentInaBJ54:

Please mention the value and describe the result.

Response:

We have revised this part to include the suggested information (line 216-217).

CommentInaBJ55:

Please omit, This section was not directly correlate with the data or result (this statement can be expressed in the introduction)

Response:

As suggested by the reviewer, we have removed this part from manuscript.

CommentInaBJ57:

This statement is more suitable in the Introduction.

Response:

We have moved this paragraph the introduction (line 42-50). However, we decided to keep line 270-275 with reason to make a link with line 275.

CommentInaBJ59:

If possible please add one paragraph regarding the possible future research, to prove whether the same result can be concluded from an in vivo study.

Response:

Thank you. We have added this information in the text (line 319-322).

CommentInaBJ60:

In this result total phenol and total flavonoid were expressed in mg GAE/g or mg RE/g but in the figure 3 they were expressed in µg/mL. why was this data and figure 3 not consistent? Please complete this data total phenol, flavonoid in µg/mL

Response:

We concur. As it appears now, Table 1 included total phenolic and flavonoid content in µg GAE/mL and µg RE/mL. We decided to keep the unit mg GAE/G dry weight and mg RE/G dry weight in the table as they have the advantage of showing the content of TPC and TFC in the starting plant material used in the experiments.

CommentInaBJ62:

Please complete the linear equation Y = a+bx and R 2 of total phenol, furthermore you could calculate the total phenolic compound of LE, FE

Response:

This information has been included. Please see the result section line 192-193.

CommentInaBJ64:

Please complete the linear equation Y = a+bx and R 2 of total flavonoid, furthermore you could calculate the total flavonoid of LE, FE

Response:

It has been addressed as suggested. Please see the result section line 194-195.

CommentInaBJ66:

Please express the complete data of Acarbose.

Please compare and check the acarbose as standard drug, why did your result has a very low activity? Please mention clearly what Journal support your result.

Response:

We appreciate the input. As suggested by the reviewer, we have added the complete inhibition % of acarbose which we obtained from triplicate experiments, please see Table 2 (line 415). As we explained previously, for the acarbose data we used reference standard acarbose from USP (United States Pharmacopeia) product. Available data acarbose activities from previous research appeared to be varied widely, for instance: 3.45 µg/ml (Ali et al., 2013), 12.6 µg/ml (David et al., 2017), 117.06 µg/ml (Mun'im et al., 2013), 1.3 mg/ml (Shukla

et al., 2016), 5 mg/ml (Kim et al., 2000), and 6.2 mg/ml (Subramanian et al., 2008). This could probably be explained from different sources of acarbose.

CommentInaBJ67:

Please explain, why did not you assay two samples in Ferrous ion chelating and one sample in DPPH free radical

Response:

We appreciate the feedback. In the present study, several methods were used to investigate the antioxidant activities of the extracts. Two of which i.e DPPH assay and ferrous ion chelating (FIC) assay are shown in Table 3. The methods are based on different reaction mechanisms. In DPPH assay, the reaction is based on scavenging mechanism of the stable radical DPPH by the donation of hydrogen and electron from the antioxidant compounds in the extracts. Reference standards BHT and ascorbic acid which undergo similar reaction were used to allow comparison (Antolovich et al., 2002). On the other hand, FIC assay is based on the chelation of Fe(II) by the antioxidant compounds in the extracts. Fe(II) is known as a catalyst in autooxidation. Reference standard for this assay is EDTA which is known as a good Fe(II) chelator. BHT and ascorbic acid are not suitable to be used as references for FIC assay because of (1) different reaction mechanism and (2) poor Fe(II) chelators due to their chemical structures. Therefore, evaluation of FIC activities for these two compounds were not applicable.

CommentInaBJ69:

If possible, please complete the figure with the standard deviation for each sample and concentration.

Response:

Thank you. As suggested by the reviewer, we have added error bars of standard deviation at every concentration level in Fig 2.

References

Ali RB, Atangwho IJ, Kuar N, Ahmad M, Mahmud R, et al. In vitro and in vivo effects of standardized extract and fractions of Phaleria macrocarpa fruits pericarp on lead carbohydrate digesting enzymes. BMC complementary and alternative medicine, 2013; 13(1): 39.

Antolovich M, Prenzler PD, Patsalides E, McDonald S, Robards K. Methods for testing antioxidant activity. Analyst, 2002; 127(1): 183-198.

David J, Afolabi EO, Ojerinde SO, Olotu PN, Agwom FM, et al. In-Vitro Antidiabetic and Antioxidant Activity of Various Leaf Extracts of Detarium microcarpum. Journal of Applied Pharmaceutical Science Vol, 2017; 7(06): 127-131.

Kim JS, Kwon CS, Son KH. Inhibition of alpha-glucosidase and amylase by luteolin, a flavonoid. Bioscience, Biotechnology and Biochemistry, 2000; 64(11): 2458-2461. doi:10.1271/bbb.64.2458

Mosier NS, Ladisch MR. 2009. Modern Biotechnology Connecting Innovations in Microbiology and Biochemistry to Engineering Fundamentals. New Jersey: John Wiley & Sons.

Mun'im A, Andriani A, Mahmudah KF, Mashita M. Screening of α-glucosidase inhibitory activity of some Indonesian medicinal plants. Int J Med Arom Plants, 2013; 3(2): 144-150.

Shukla S, Park J, Kim D-H, Hong S-Y, Lee JS, et al. Total phenolic content, antioxidant, tyrosinase and α-glucosidase inhibitory activities of water soluble extracts of noble starter

culture Doenjang, a Korean fermented soybean sauce variety. Food Control, 2016; 59: 854-861. doi:http://dx.doi.org/10.1016/j.foodcont.2015.07.003
Subramanian R, Asmawi MZ, Sadikun A. In vitro alpha-glucosidase and alpha-amylase enzyme inhibitory effects of Andrographis paniculata extract and andrographolide. Acta Biochim Pol, 2008; 55(2): 391-398.
Surya S, Salam AD, Tomy DV, Carla B, Kumar RA, et al. Diabetes mellitus and medicinal plants-a review. Asian Pacific Journal of Tropical Disease, 2014; 4(5): 337-347.
Yilmazer-Musa M, Griffith AM, Michels AJ, Schneider E, Frei B. Inhibition of α-amylase and α-glucosidase activity by tea and grape seed extracts and their constituent catechins. J Agric Food Chem, 2012; 60(36): 8924.

Response to Revisions Request (Review Result Round 2)

M201743

Dear Editors

Our sincerest thanks for the opportunity to revise our manuscript titled "In Vitro Antidiabetic and Antioxidant Activities of Aqueous Extract from the Leave and Fruit of *Psidium guajava* L" in your journal. We also thank the Reviewer for the careful reviews and helpful suggestions. We believe these have resulted in improvement of our manuscript. Below are our responses to the requested revisions.

We have made changes as requested and wherever appropriate. Changes were marked in blue highlight in the manuscript. The revision was developed in consultation with all co-authors and each author has given approval to the final form of this revision.

CommentInaBJ1:

This result was not competitive, this result showed that acarbose was the lowest α-glucosidase inhibition activity compared to LE, FE. Please change this statement.

Response:

Thank you. We appreciate the comment. We noticed that the referred sentences in the abstract was also asked in the review round 1 and we have reworded the statement into the current one.

The word “competitive” in the abstract referred to the mode or type of enzyme inhibition. It has been known that reactions that involve enzymes can be inhibited by certain substances, giving rise to a reduced velocity of an enzyme reaction (Mosier & Ladisch, 2009). There are several inhibition mechanisms, *i.e* competitive, non-competitive, and un-competitive. One way to determine which inhibition mode take place is by conducting a kinetic experiment to the said reaction. A double reciprocal graph between 1/[S] vs 1/[V] was generated, and the inhibition mechanism was analysed from the pattern of the graph produced. This method is known as a Lineweaver-Burke plot analysis (Lineweaver & Burk, 1934).

In our study, inhibition mechanisms for the inhibition of both our sample and acarbose against α-glucosidase were determined. Results showed that the inhibition graphs produced by our sample leaf extract (LE) and by a synthetic inhibitor (acarbose) exhibited a similar inhibition mechanism (Figure 2). Both indicated a competitive inhibition type which means that the inhibitors compete with the substrate for the enzyme active site.

In order to better represent this explanation and to avoid ambiguous interpretation, we have revised the sentences as follows:

Statement in line 12-13 in the abstract:

The enzyme kinetic analysis indicated that LE inhibited α-glucosidase in a **competitive** mode, **similar to** that of acarbose.

has been amended into:

The enzyme kinetic analysis indicated that LE inhibited α-glucosidase in a **competitive inhibition type**, **similar to** that of acarbose.

CommentInaBJ2:

Please explain more about α-glucosidase inhibitory activity toward diabetes.

Response:

The requested suggestion has been added to the text. We incorporated the sentences in line 32-37 to maintain good flow of explanation.

CommentInaBJ3, and the same comment 4,5,6 and 7:

Please write the exact concentration of gallic acid, just use comma to separate each concentration if you used different concentration (do not write in range).

Response:

Throughout the manuscript, changes have been made to include exact concentrations used in the experiments, i.e line 93, line 112-113, line 137-139, line 152-153, and line 154.

CommentInaBJ8

Please write the exact concentration of gallic acid, just use comma to separate each concentration if you used different concentration (do not write in range).

Response:

Range of concentrations which appeared previously in line 207 has been deleted because of repetition of information.

CommentInaBJ9

Please mention the acarbose values based on previous studies when it is compared to other plant extracts.

Response:

Several previous studies have been included as appeared in line 267 – 275 for the comparison of α-glucosidase inhibitory activities of plant extracts and acarbose.

Comment 10: Conflict of interest

Response:

Statement regarding **Conflict of interest** has been added, line 338.

Comment 11: Acknowledgement

Response:

Statement regarding **Acknowledgement** has been added, line 340-342.

References

Lineweaver H, Burk D. The determination of enzyme dissociation constants. J Am Chem Soc, 1934; 56: 658-666.

Mosier NS, Ladisch MR. 2009. Modern biotechnology connecting innovations in microbiology and biochemistry to engineering fundamentals. New Jersey: John Wiley & Sons.

Gmail M201743

8 of 12

[InaBJ] M201743 Editor Decision - Revisions Required

Inbox ×

Secretariat of The Indonesian Biomedical Journal <secretariat@inabj.org>
to me, ferrysandra

Tue, Jan 2, 2018, 4:31 PM

Dear Dr. Adelina Simamora,

Good day. We have reached a decision regarding your submission to The Indonesian Biomedical Journal, "**In Vitro Antidiabetic and Antioxidant Activities of Aqueous Extract from the Leave and Fruit of Psidium guajava L**".

This manuscript is interesting, but it **needs to be corrected** before it can be published in The Indonesian Biomedical Journal. You can find the reviewers' comments and suggestions on the manuscript attached.

Please revised this manuscript according to reviewers' suggestions. Provide us an added/corrected/revised version of your manuscript and also a response letter to reviewer before **January 22, 2018**. Please mark/highlighted the revised part of the manuscript, so that editor will notice the changes.

When you done, you can upload it in: https://inabj.org/index.php/ibj/author/submissionReview/402, or simply email us. Feel free to make justification and also provide us your comments if you found inapplicable comments or suggestions.

Please let us know if you have any questions.
Thank you for your attention. We wish you a nice day.

Gmail M201743

6 of 12

[InaBJ] M201743 Editor Decision - Minor Revision

Inbox ×

Secretariat of The Indonesian Biomedical Journal <secretariat@inabj.org>
to me

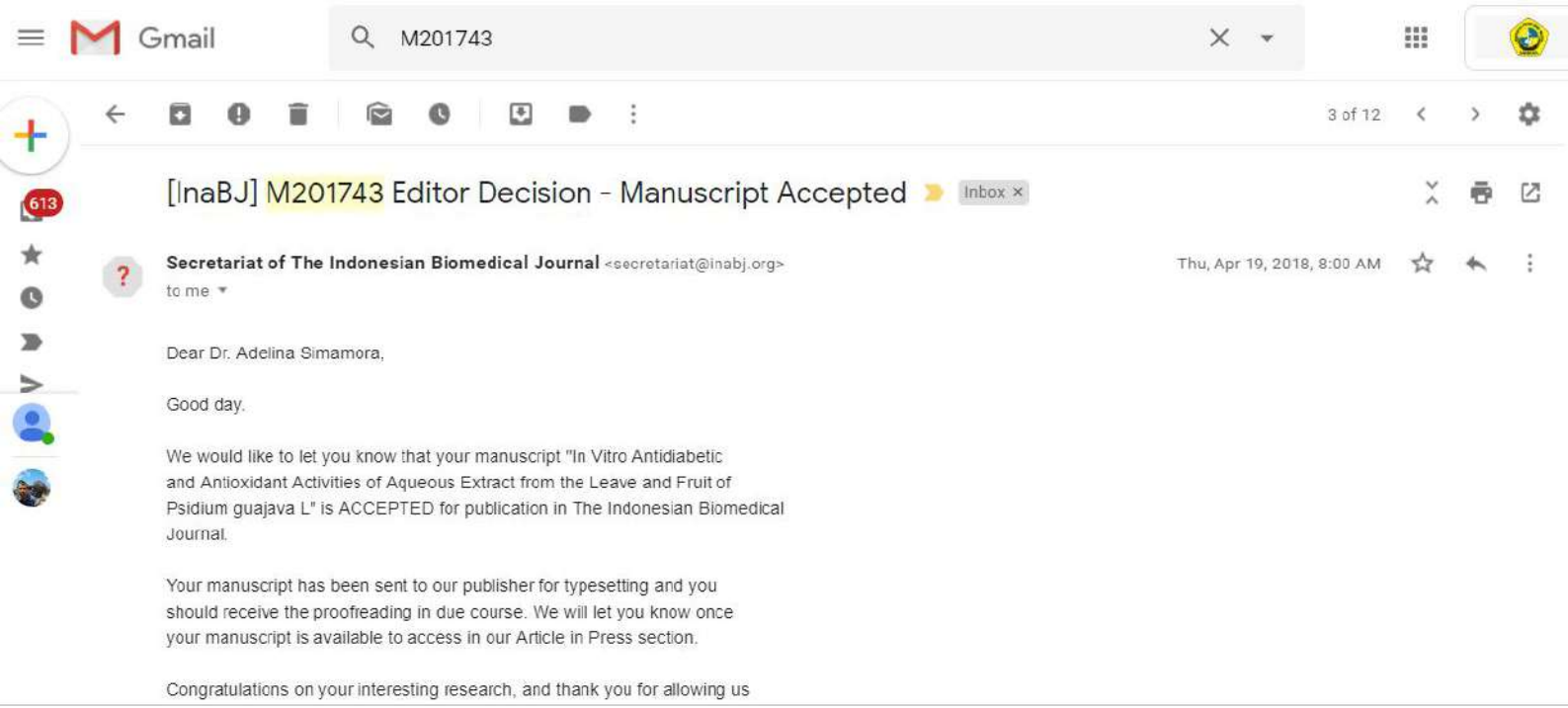
Thu, Mar 22, 2018, 3:15 PM

Dear Dr. Adelina Simamora,

Good day. We have reached a decision regarding your submission to The Indonesian Biomedical Journal, "In Vitro Antidiabetic and Antioxidant Activities of Aqueous Extract from the Leave and Fruit of Psidium guajava L". Our decision is: Revisions Required.

You did a great work in revising the manuscript previously, but it needs to some minor revision before it can be published in The Indonesian Biomedical Journal. Please find our reviewer's comments on the manuscript attached.

Please provide us a revised version of your manuscript before April 6, 2018. Please feel free to make justification and also provide us your comments if you found inapplicable comments. Please let us know if you have any


Gmail
M201743
3 of 12
[InaBJ] M201743 Editor Decision - Manuscript Accepted
Inbox ×
Secretariat of The Indonesian Biomedical Journal <secretariat@inabj.org>
to me
Thu, Apr 19, 2018, 8:00 AM
Dear Dr. Adelina Simamora,
Good day.
We would like to let you know that your manuscript "In Vitro Antidiabetic and Antioxidant Activities of Aqueous Extract from the Leave and Fruit of Psidium guajava L" is ACCEPTED for publication in The Indonesian Biomedical Journal.
Your manuscript has been sent to our publisher for typesetting and you should receive the proofreading in due course. We will let you know once your manuscript is available to access in our Article in Press section.
Congratulations on your interesting research, and thank you for allowing us

Gmail M201743

1 of 12

M201743 Proof Reading final correction and approval by author

adelina simamora <adelina.simamora@ukrida.ac.id>
to Secretariat, bcc: adelina_1512

Mon, Jun 25, 2018, 12:52 PM

Dear Editor

Please find attached our final correction and form of proof reading approval by author for our manuscript titled:
"***In Vitro* Antidiabetic and Antioxidant Activities of Aqueous Extract from the Leave and Fruit of *Psidium guajava* L."**

Thank you for the opportunity to publish our work in your platform. We are looking forward to send more of our research to your journal.

Best regards

Adelina Simamora et al

2 Attachments