January 10, 2022

Dear Editor,

I would like to submit a manuscript entitled “Determination of the active chemical compounds and the antibacterial activity of various fractions of *Lawsonia inermis* L. to be consider for publication as “an original article” in Borneo Journal of Pharmacy.

The antibacterial activity of henna leaves extract in various solvents has been studied in a number of investigations. Although *L. inermis* has been found to have antibacterial action, it is yet unknown which component is responsible for the antibacterial activity. In this work, three distinct fractions of henna leaves were tested against *S. aureus*, and TLC-bioautography was used to identify the class of active chemicals as antibacterial agents from the most active fraction.

We declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

We know of no conflict of interest associated with this publication, there has been no significant financial support for this work that could have influenced its outcome.

Sincerely,

Sri Mulyaningsih

May 7, 2022

Dear Editor

I hereby resubmit the corrected manuscript entitled "Determination of the active chemical compounds and the antibacterial activity of various fractions of *Lawsonia inermis* L." for another review.

We have corrected our manuscript according to the comments from the reviewer. The following is a list of comments and corrections given to the manuscript. Please find it at the end of this letter. If anything needs to be corrected, please contact us again.

Thank you for considering this manuscript to be published in the Borneo Journal of Pharmacy.

Best regards

Sri Mulyaningsih

List of comments and corrections given to the manuscript.

Manuscript title
Determination of the Active Chemical Compounds and the Antibacterial Activity of Various Fractions of *Lawsonia inermis* L.

Comments on the **introduction**

Comments	Corrections
1. Lawsonia inermis or well known as henna → where is it known?	1. It has been corrected in the manuscript
2. Henna leaves are reported to contain large amounts of chemical compounds such as **lawson** → you mean lawsone? Lawson is a convenience store	2. It has been corrected in the manuscript
3. In this study, the methanol extract of henna leaves was fractionated with n-hexane, ethyl acetate to obtain n-hexane, ethyl acetate and methanol fractions → Authors must explain the reasons for choosing the solvent for each fraction	3. The reason has been explained in the manuscript
4. Why did the authors only determine the group, not the identity and structure of the active compound, even though it was already difficult to do fractionation?	4. The reason has been explained in the manuscript
5. The objective of this study was to **know** the antibacterial activity of the active fraction of henna leaves and to determine the class of active compounds as antibacterial agent from the most active fraction against S.aureus. → to know or also to determine	5. It has been corrected in the manuscript

Comments on **methods**

Comments	Corrections
1. Kindly state the voucher number of the determined specimen	1. It has been corrected in the manuscript
2. Also report the instrument used	2. It has been corrected in the manuscript
3. A sterile cotton swab was used to apply the S.aureus bacterium suspension to the agar surface → What is the concentration of the bacterial inoculum?	3. It has been added in the manuscript (1 x 10^8 cfu/ml)
4. After that, the silica gel plate was placed on the surface of the MHA agar medium in an inverted position and left for 30 minutes → How do authors determine contact times for autobiographies?	4. The contact time for bioautography was determined based on the journal as well as our trial/orientation.

Comments on **results and discussion**

Comments	Corrections

1. In Table I, how can the methanol extract provide the largest inhibition zone, but its fraction does not provide any inhibition at all (0)? Authors should discuss these interesting findings, not dismiss them as if they were not interesting.	1. It has been discussed in the manuscript
2. Still in Table I, if the methanol fraction does not produce an inhibition zone, why is it written (-) instead of 0 like DMSO?	2. It has been corrected in the manuscript
3. In Table II, the value of the inhibition zone in the fraction with a concentration of 10% is exactly the same as in Table I. Does that mean the authors used the previous data? If so, it means that the authors' statement "The concentrations of ethyl acetate fraction tested were 5, 10, 15, and 20 %w/v" is incorrect because the 10% concentration was not repeated. Supposedly, the authors repeated the test again	3. It has been corrected in the manuscript. (The 10% has been removed)
4. What do the + and - symbols in Table III mean?	4. It has been explained in the manuscript
5. Figure 1 should be separated into 2 different figures, with the bioautography results presented separately, because it is important to see the zone of inhibition. In addition, there is no explanation of what the black circle in the picture means	5. The figure 1 has been separated into 2 figures

Comments on conclusion

Comments	Corrections
These results indicate that the ethyl acetate fraction of henna leaves contains naphtoquinone, flavonoid, and phenolic compounds (tannins) which have the ability to inhibit bacterial growth → Where did the authors conclude that the phenolic compounds in this fraction were tannins? Certain flavonoids can also give results on the FeCl3 test because they have a phenol group	It has been corrected in the manuscript.

Please write another review that has not been covered above.

Comments	Corrections
Also, add Funding details, Data availability, and Authors contributions (according to the guidelines and templates)	They have been added on the manuscript

July 19, 2022

Dear Editor

Herein I am submitting the corrected manuscript entitled "Determination of the active chemical compounds and the antibacterial activity of various fractions of *Lawsonia inermis* L." for minor revision.

We have corrected our manuscript according to the comments from the reviewer. The following is a list of comments and corrections given to the manuscript. Please find it at the end of this letter.

Thank you for considering this manuscript to be published in the Borneo Journal of Pharmacy.

Best regards

Sri Mulyaningsih

List of comments and corrections given to the manuscript.

Manuscript title

Determination of the Active Chemical Compounds and the Antibacterial Activity of Various Fractions of *Lawsonia inermis* L.

Comments on the **introduction**

Comments	**Corrections**
This study only determined the class of compounds from antibacterial active compounds, not to identify any of these active compounds because isolation, purification, and identification stages are needed and an adequate amount of isolates is required for the purpose of identifying the structure of the isolates → If the limitations of this study and the additional studies needed are known, why was this additional study not carried out by increasing the amount of extract to obtain sufficient isolates?	This sentence was moved to the end of the discussion. As this is the weakness of our reasearch, we suggest for the further investigation.

Comments on **results and discussion**

Comments	**Corrections**
1. is remarkable that the methanol extract had the biggest inhibitory zone, but the methanol fraction had none. There are alternatives that could account for this outcome. First, a greater methanol fraction concentration is required to inhibit S. It aureus. The tested concentration of 10% methanol fraction did not offer inhibition against S. aureus in this test. It is possible that the methanol fraction will give inhibition if the concentration is raised. Second, because the antibacterial active chemicals in henna leaves are	1. It has been discussed in the manuscript

likely to be non-polar or semi-polar, they have been separated into nhexane and ethyl acetate fractions. The antibacterial activity of the methanol fraction was reduced as a result → The first reason is unlikely to occur, because even if the concentration is too low, there should still be an inhibition zone even though it is very small. While the second reason is also dubious, because if the active compound has been extracted by n hexane or ethyl acetate, why is the inhibition zone of the two not larger than the methanol extract? 2. In Figures 1 and 2, also include the Petri dishes from the bioautography so that there is evidence of the resulting inhibition zone	2. Figure 1 and 2 have been added the petridishes.