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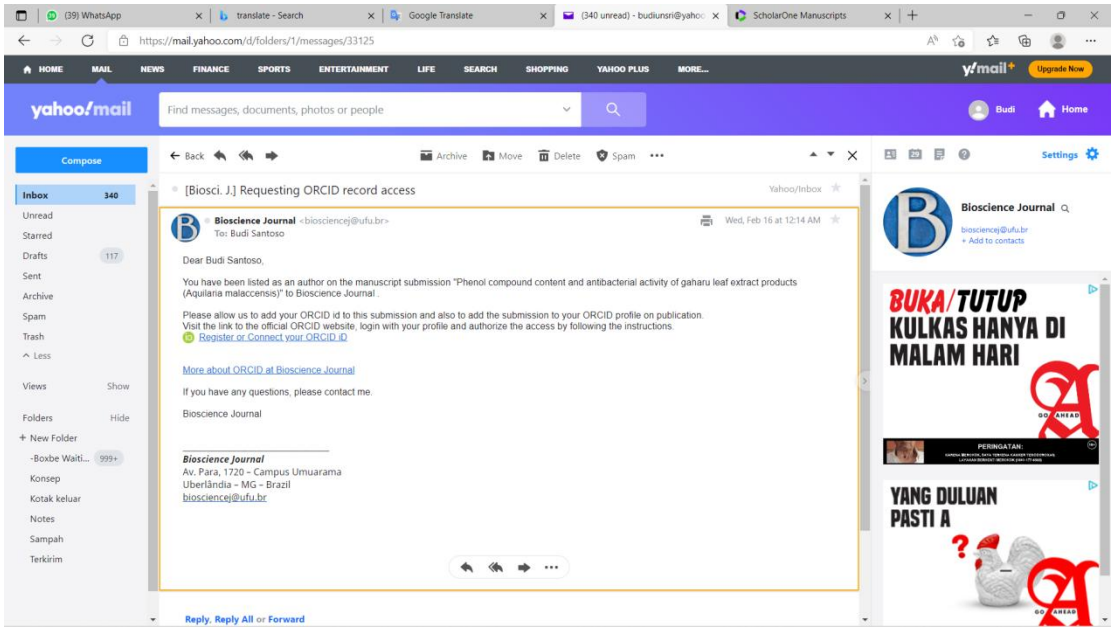

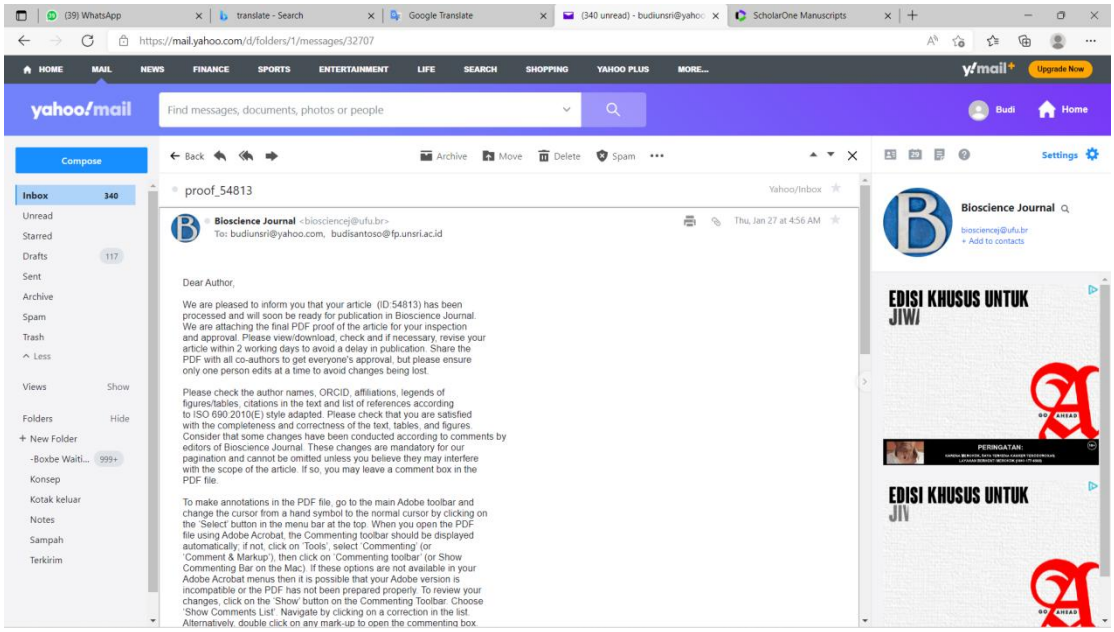

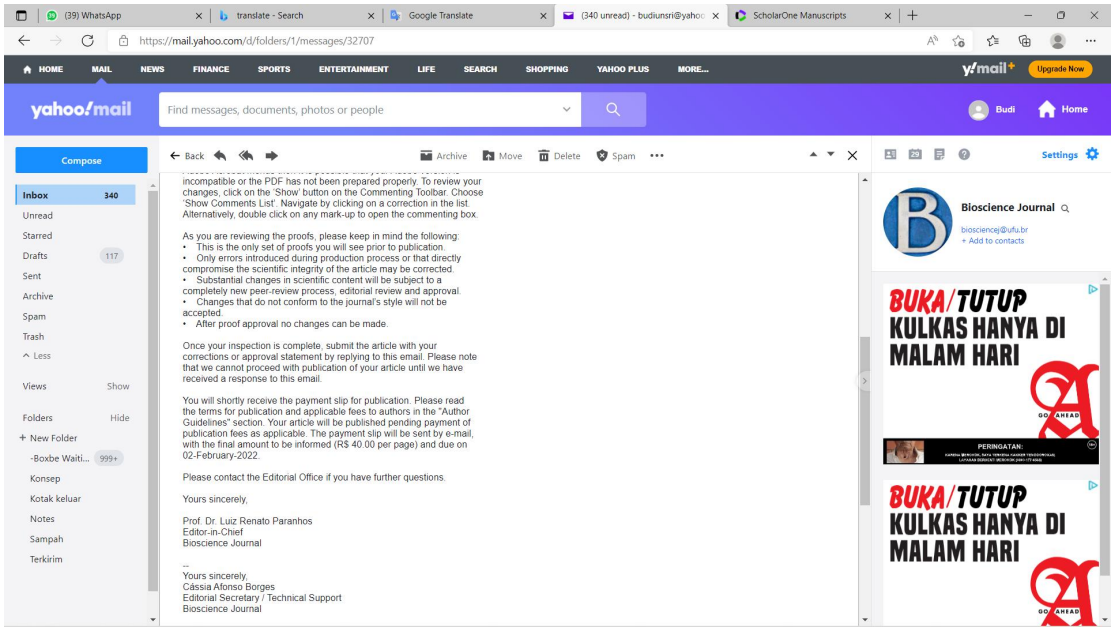
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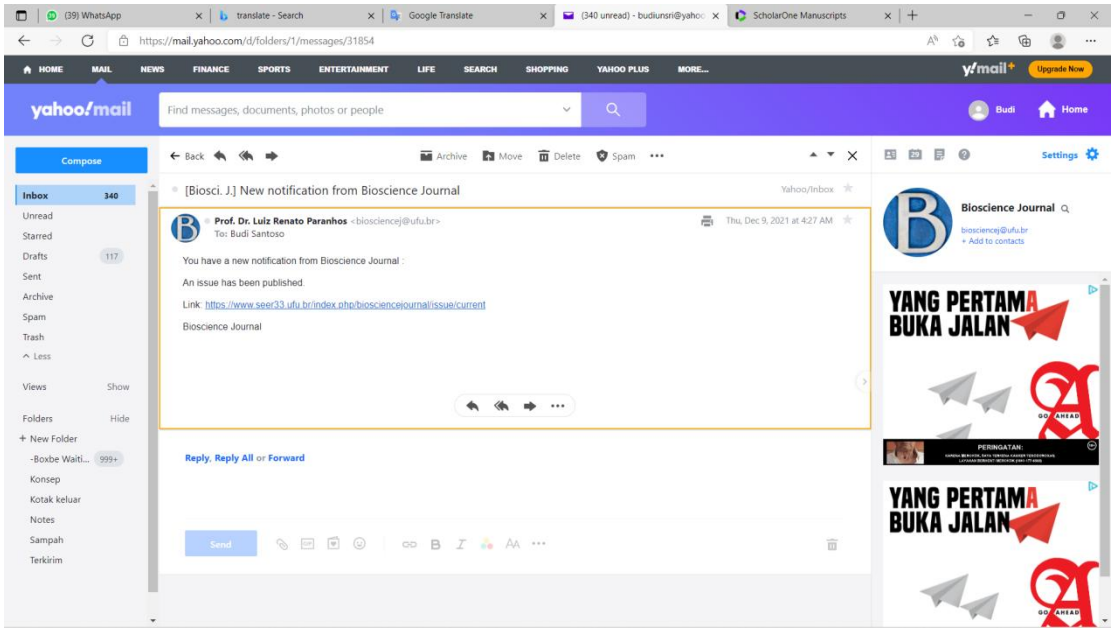
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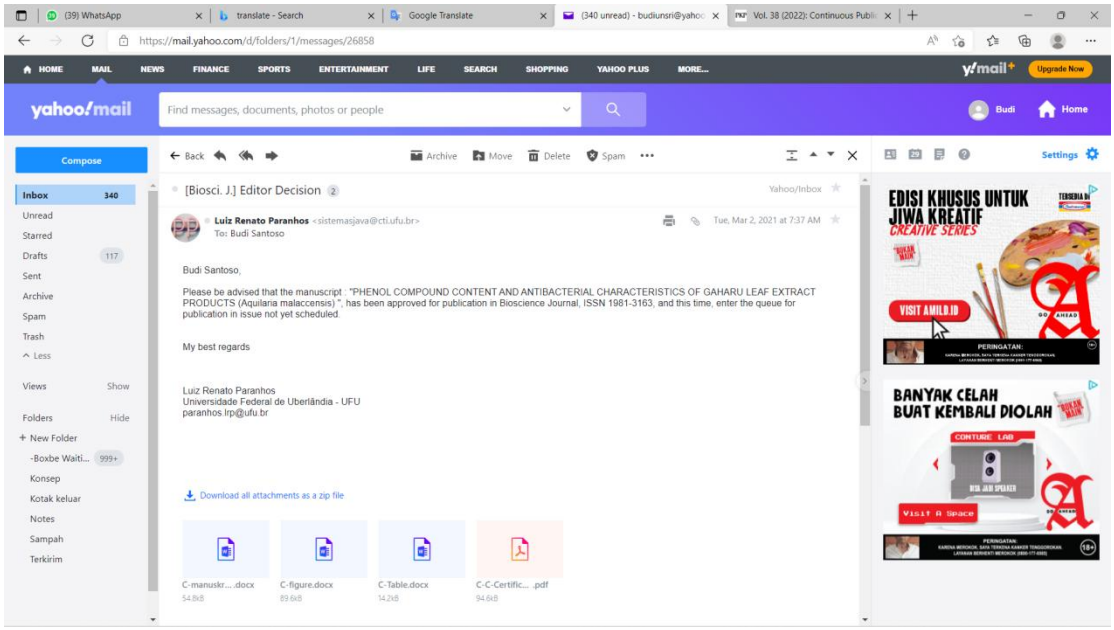
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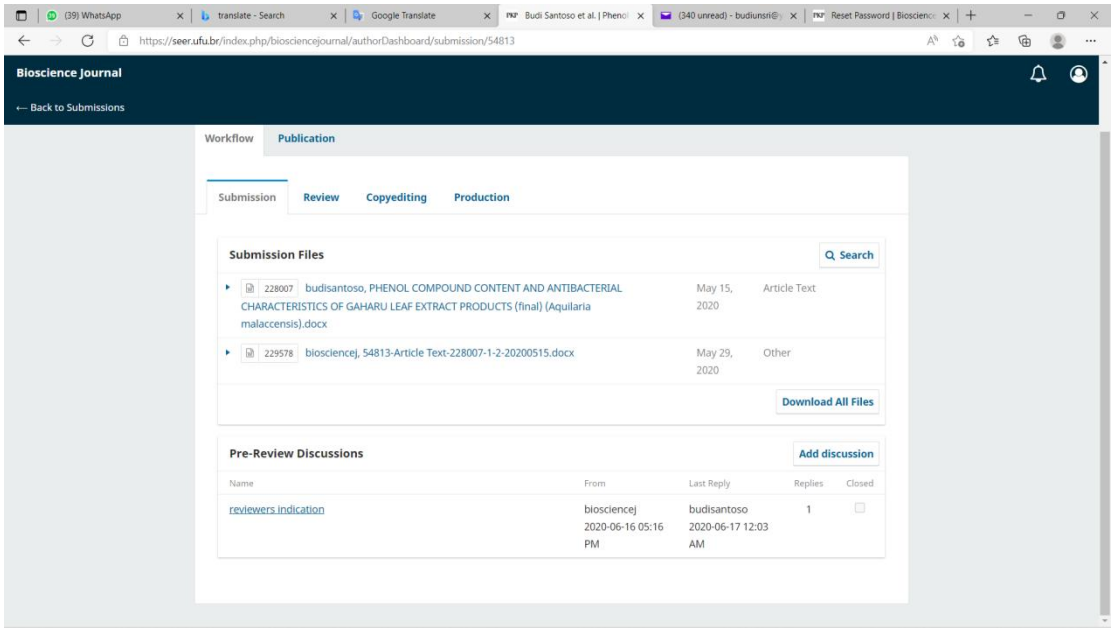
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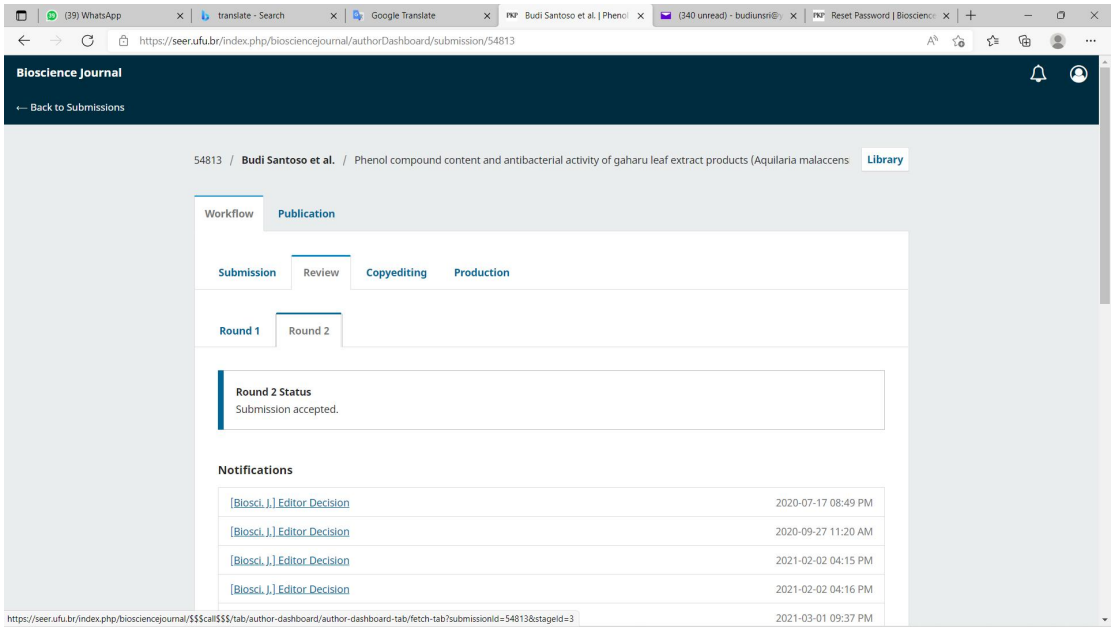
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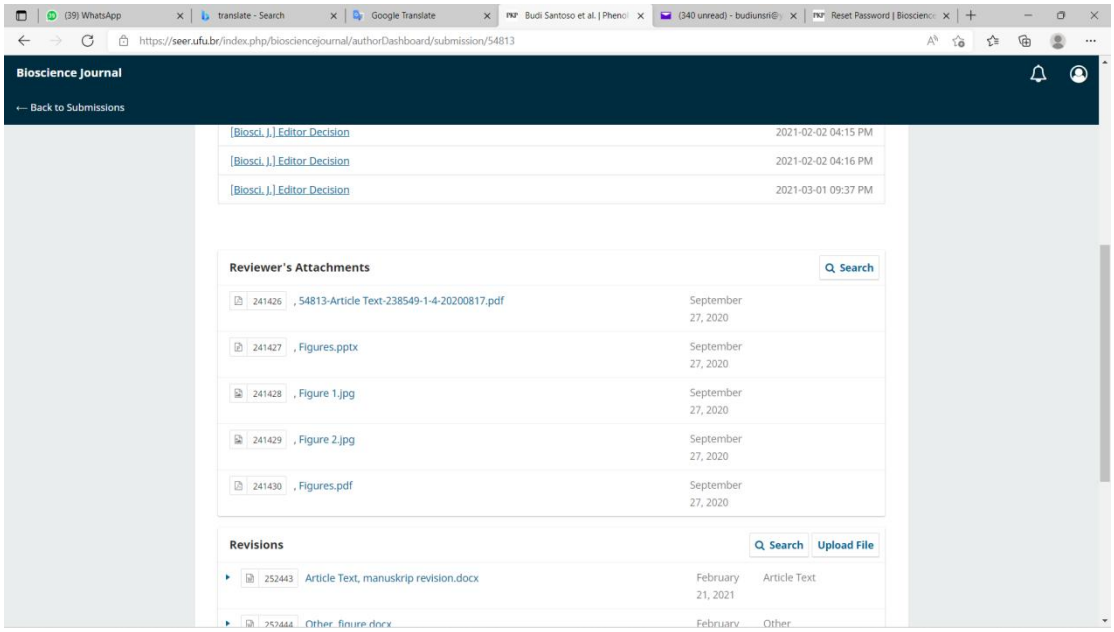
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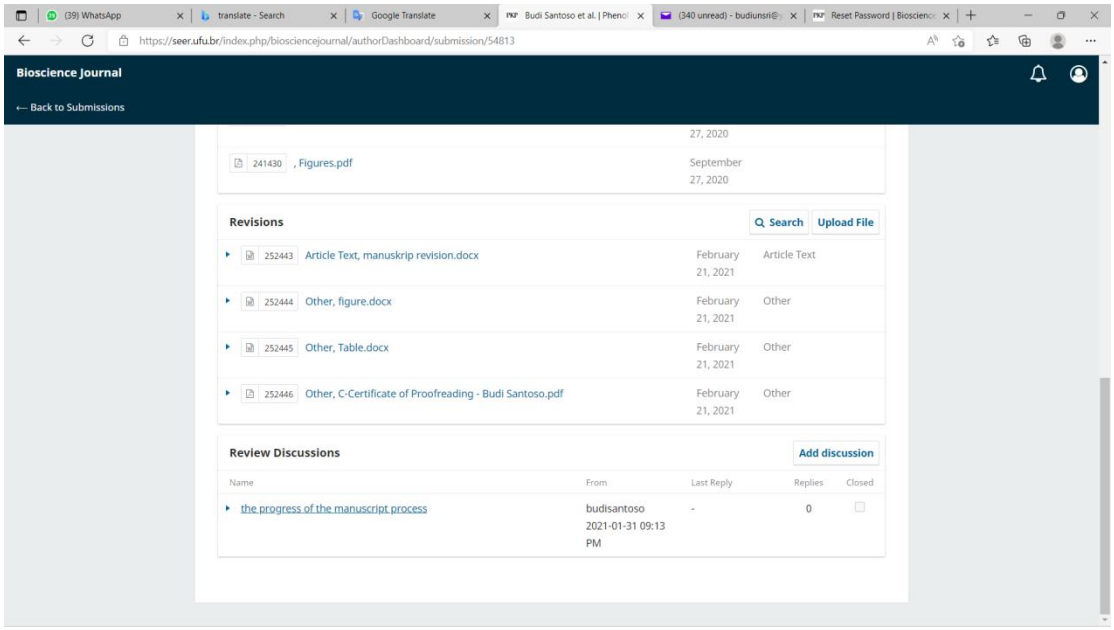
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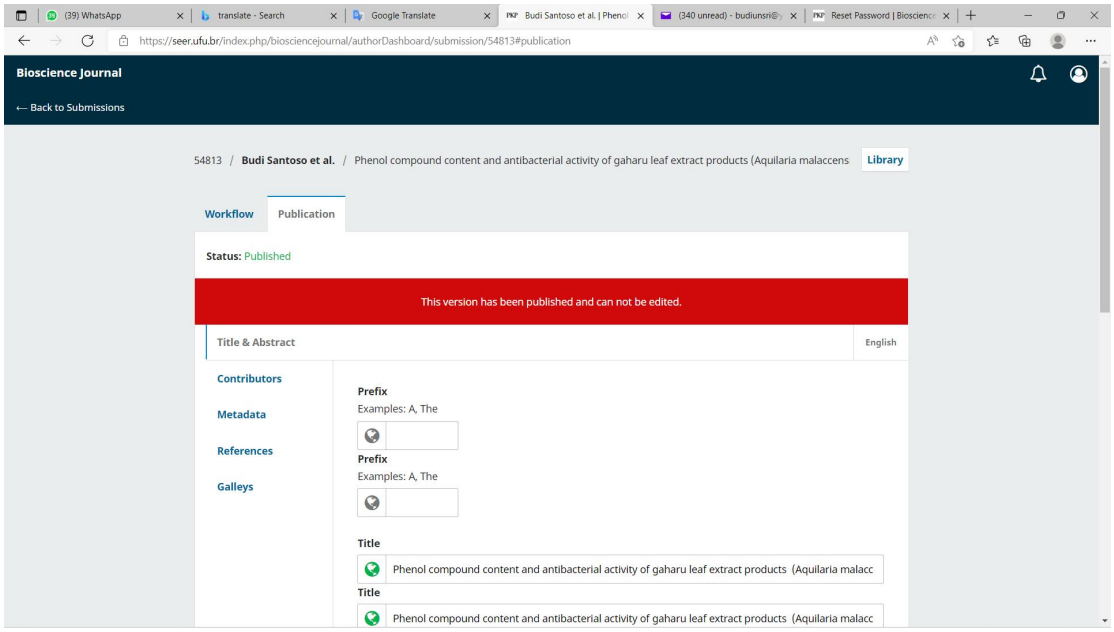
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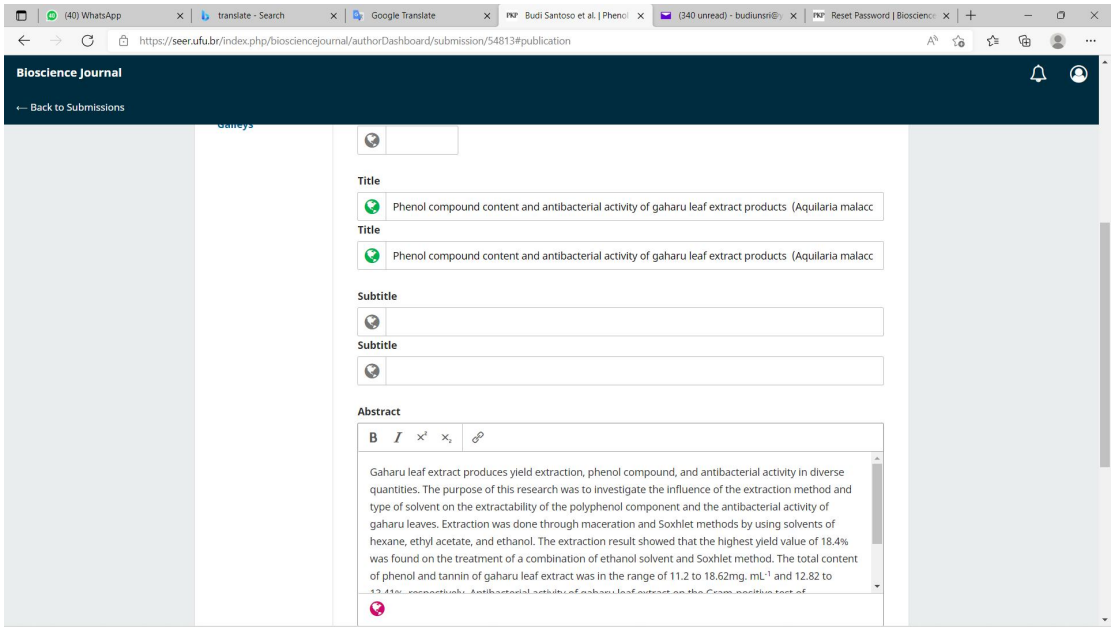
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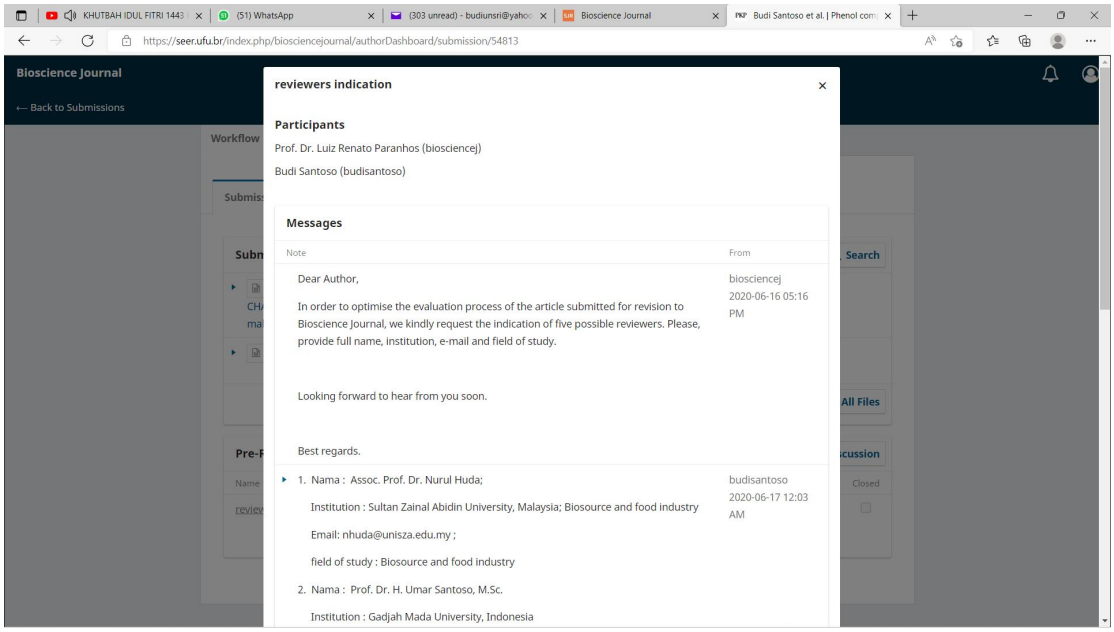
Abstract
Gaharu leaf extract produces yield extraction, phenol compound, and antibacterial activity in diverse quantities. The purpose of this research was to investigate the influence of the extraction method and type of solvent on the extractability of the polyphenol component and the antibacterial activity of gaharu leaves. Extraction was done through maceration and Soxhlet methods by using solvents of hexane, ethyl acetate, and ethanol. The extraction result showed that the highest yield value of 18.4% was found on the treatment of a combination of ethanol solvent and Soxhlet method. The total content of phenol and tannin of gaharu leaf extract was in the range of 11.2 to 18.62mg. mL-1 and 12.82 to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Budi Santoso (budisantoso)
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Institution : Sultan Zainal Abidin University, Malaysia; Biosource and food industry
Email: nhuda@unisza.edu.my ;
field of study : Biosource and food industry
2. Nama : Prof. Dr. H. Umar Santoso, M.Sc.
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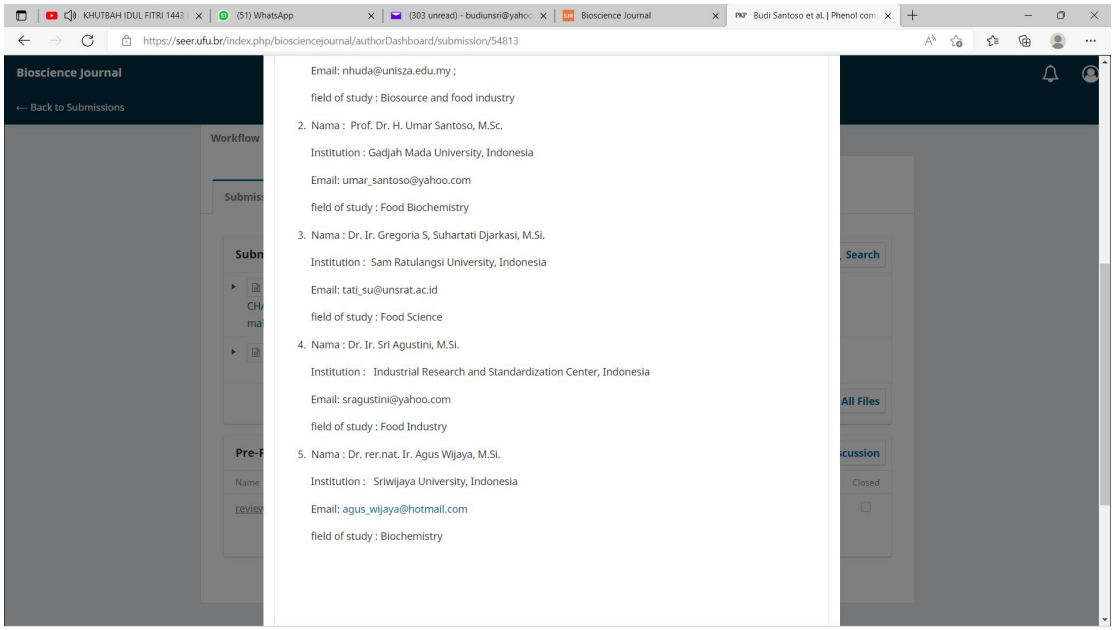

Email: nhuda@unisza.edu.my ;

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2. Nama : Prof. Dr. H. Umar Santoso, M.Sc.

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Email: umar_santoso@yahoo.com

field of study : Food Biochemistry

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Institution : Sam Ratulangsi University, Indonesia

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field of study : Food Science

4. Nama : Dr. Ir. Sri Agustini, M.Si.

Institution : Industrial Research and Standardization Center, Indonesia

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field of study : Food Industry

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Institution : Sriwijaya University, Indonesia

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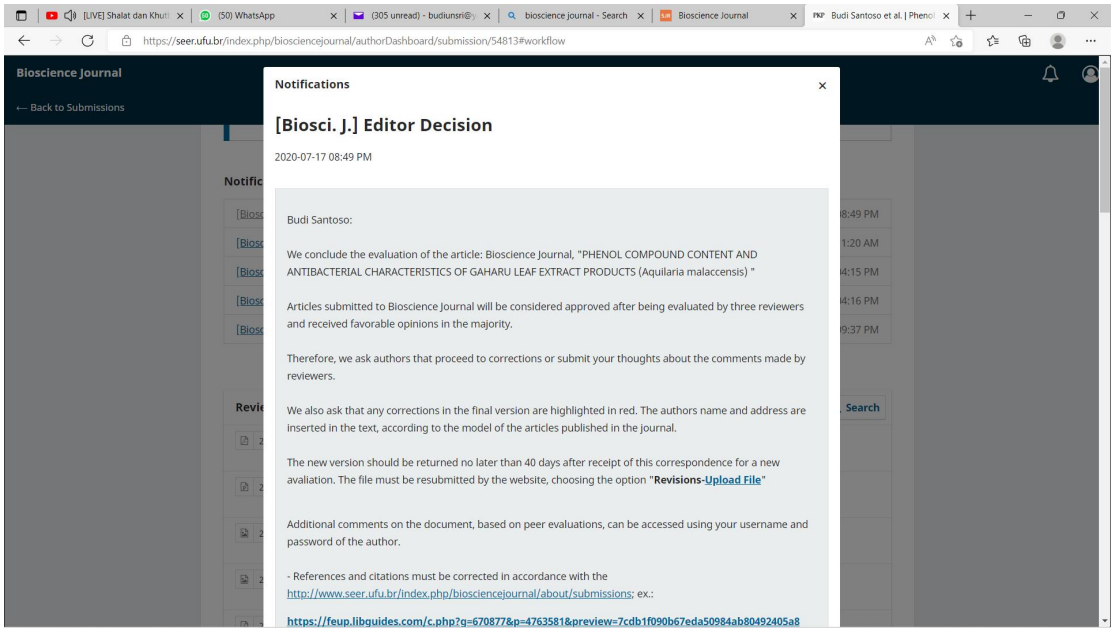

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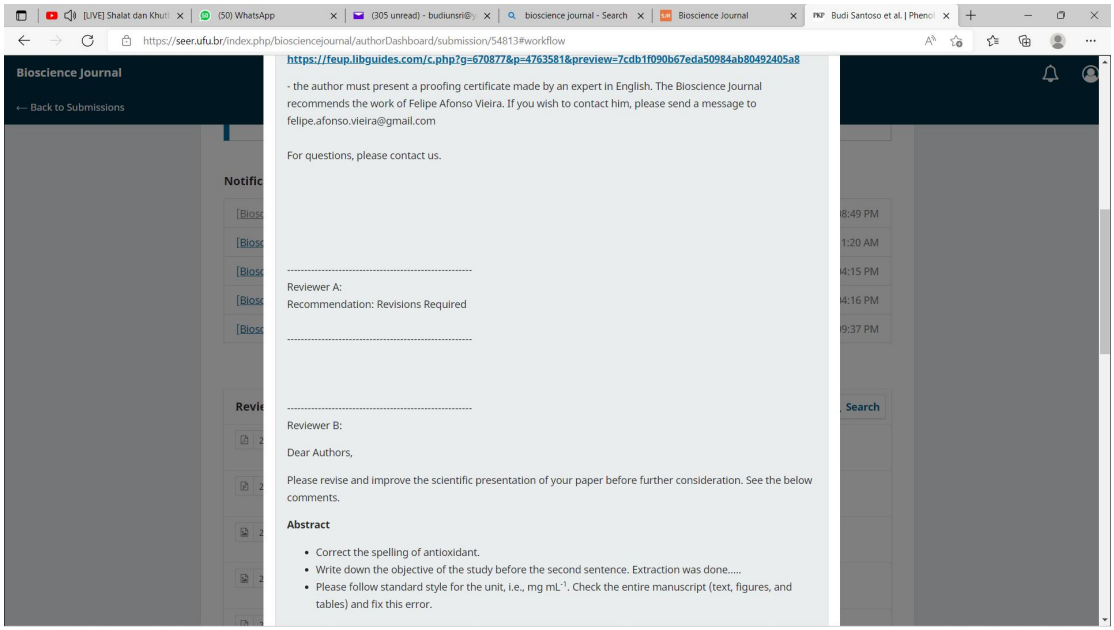
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• Correct the spelling of antioxidant.
• Write down the objective of the study before the second sentence. Extraction was done.....
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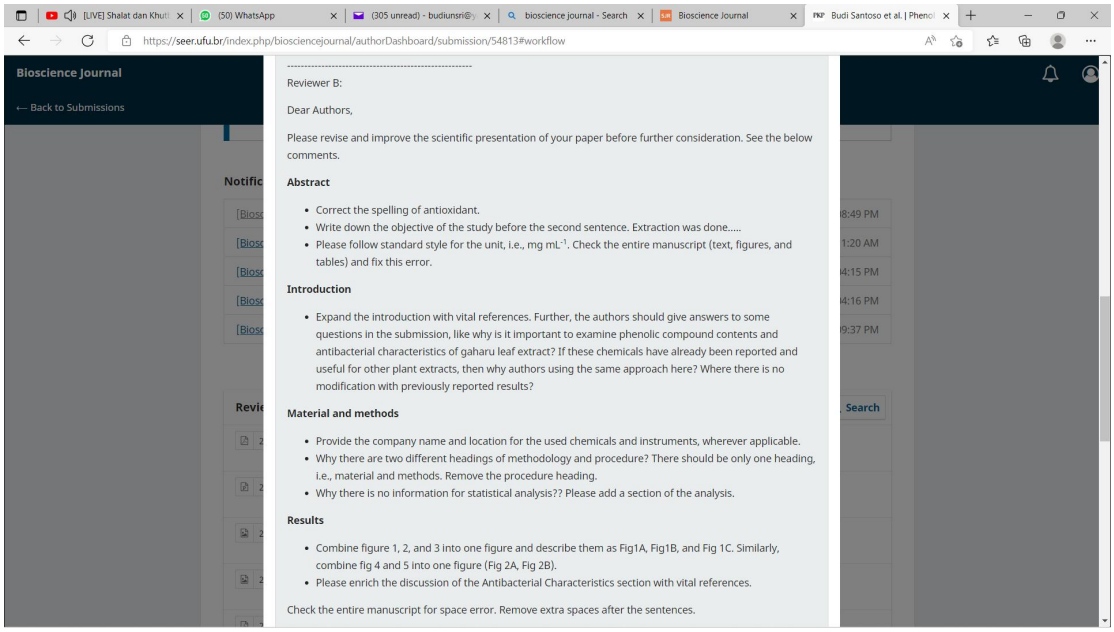
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Material and methods
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Results
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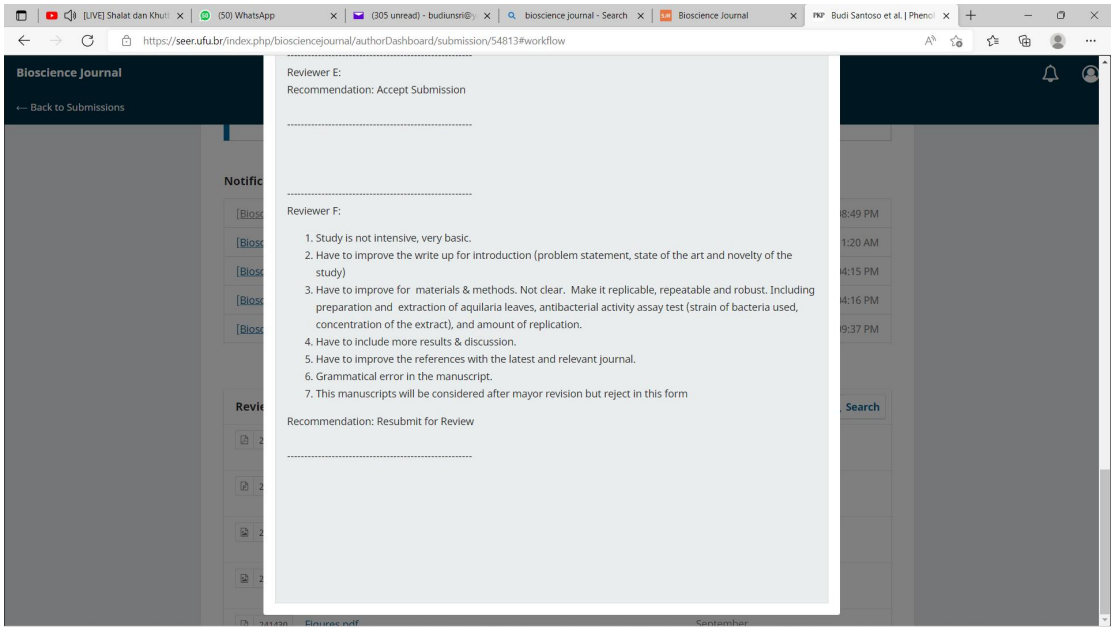
Check the entire manuscript for space error. Remove extra spaces after the sentences.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Reviewer F:
1. Study is not intensive, very basic.
2. Have to improve the write up for introduction (problem statement, state of the art and novelty of the study)
3. Have to improve for materials & methods. Not clear. Make it replicable, repeatable and robust. Including preparation and extraction of aquilaria leaves, antibacterial activity assay test (strain of bacteria used, concentration of the extract), and amount of replication.
4. Have to include more results & discussion.
5. Have to improve the references with the latest and relevant journal.
6. Grammatical error in the manuscript.
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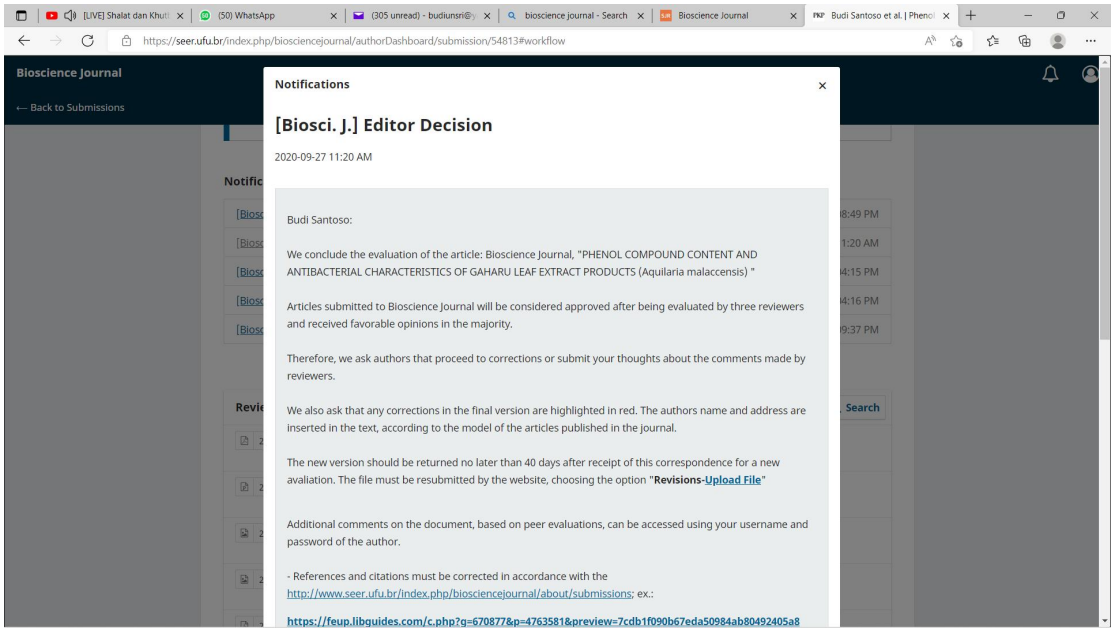
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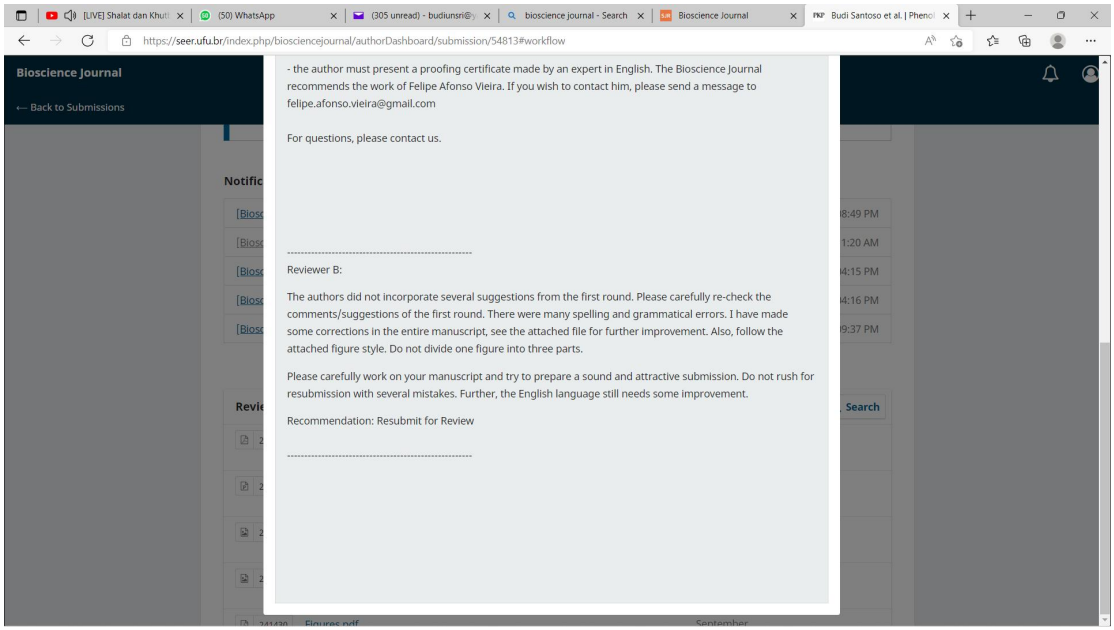
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The authors did not incorporate several suggestions from the first round. Please carefully re-check the comments/suggestions of the first round. There were many spelling and grammatical errors. I have made some corrections in the entire manuscript, see the attached file for further improvement. Also, follow the attached figure style. Do not divide one figure into three parts.
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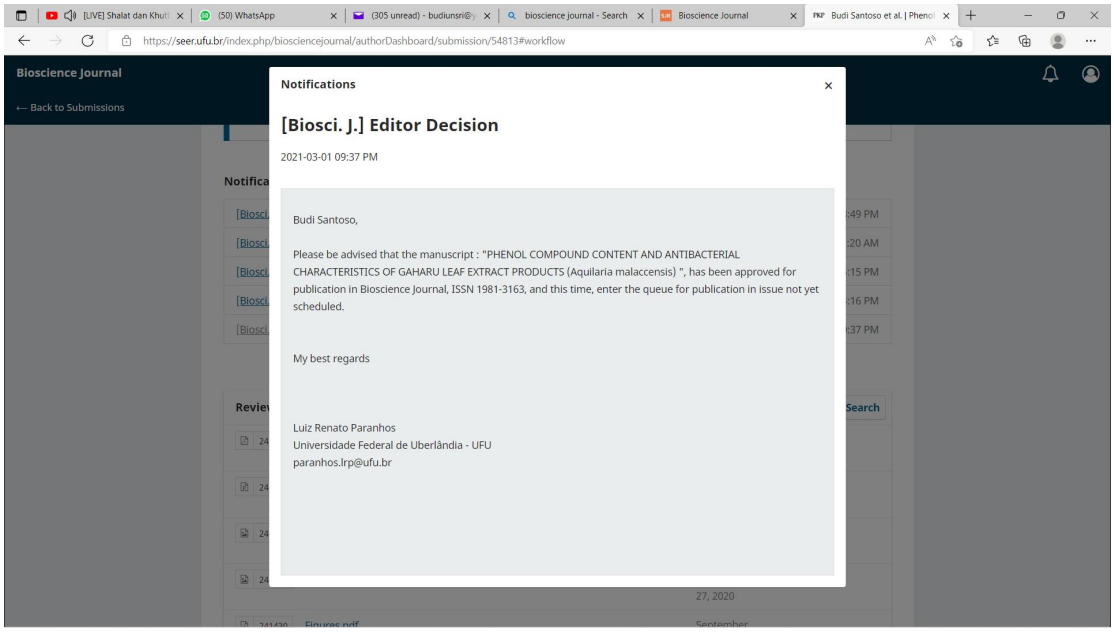
Recommendation: Resubmit for Review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My best regards
Luiz Renato Paranhos
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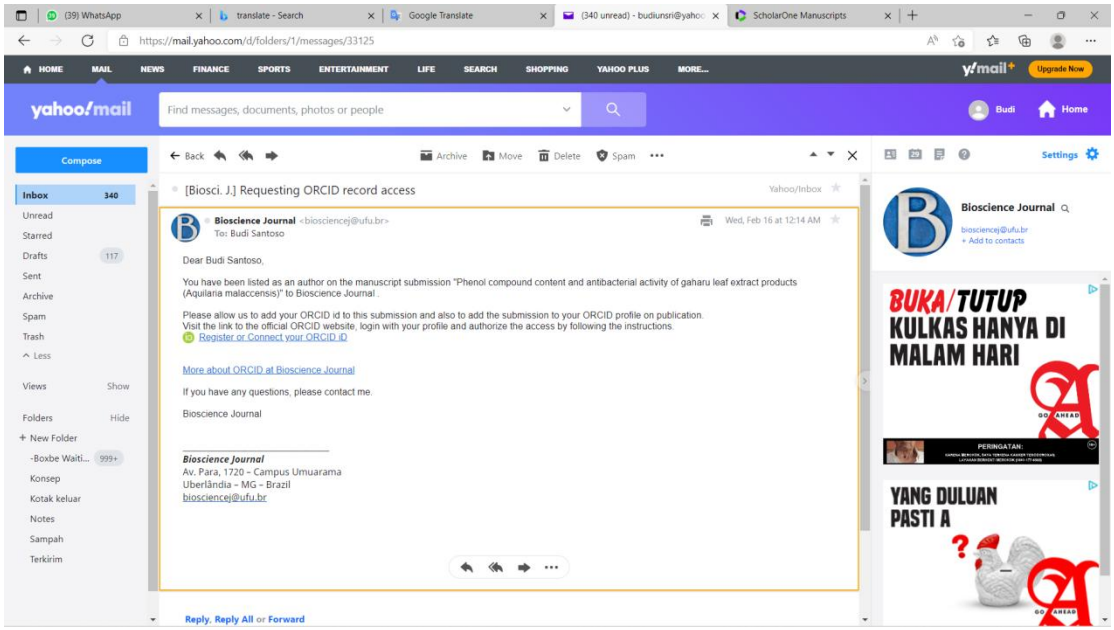

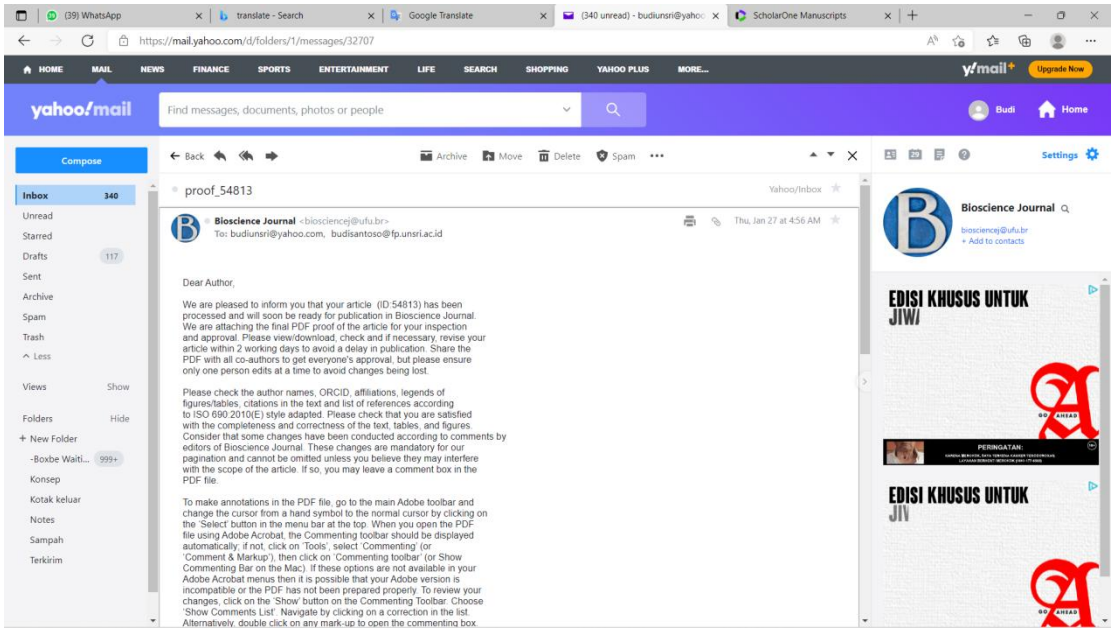

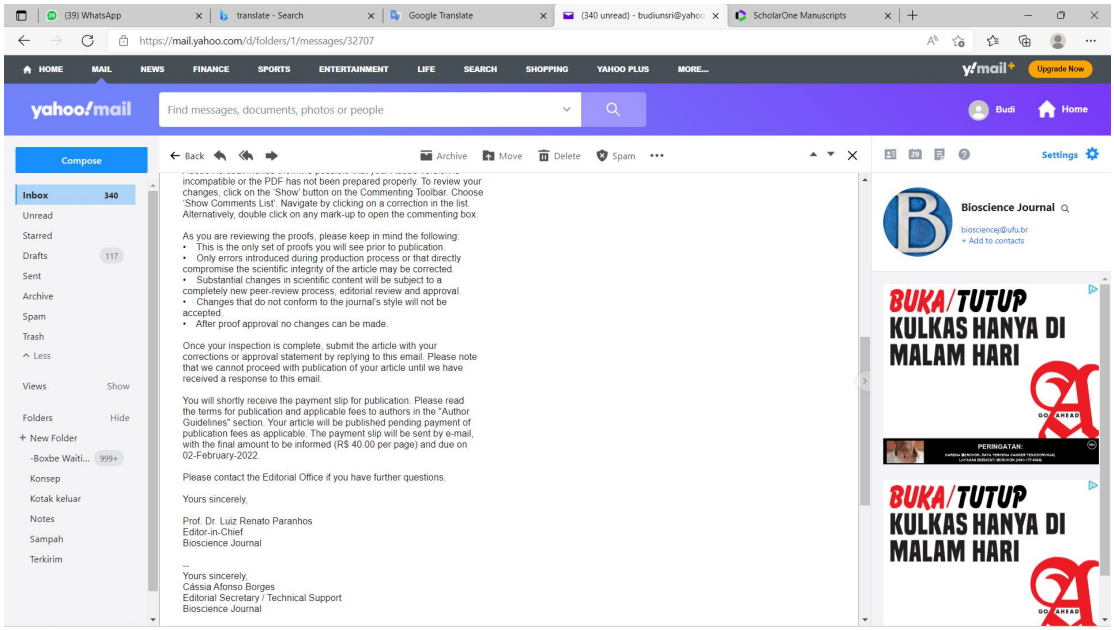
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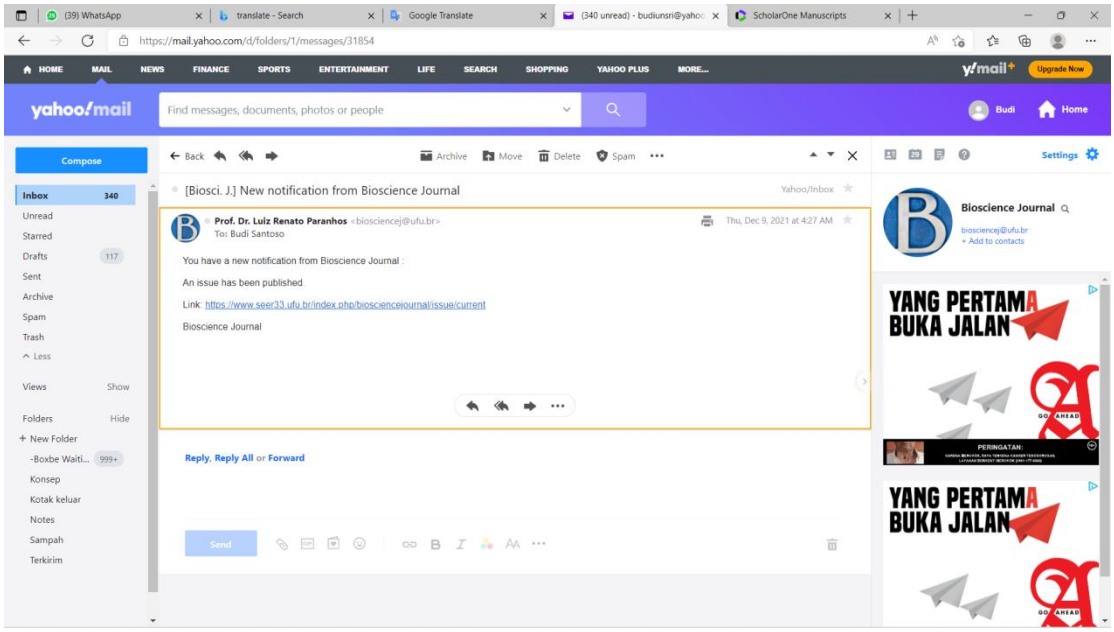
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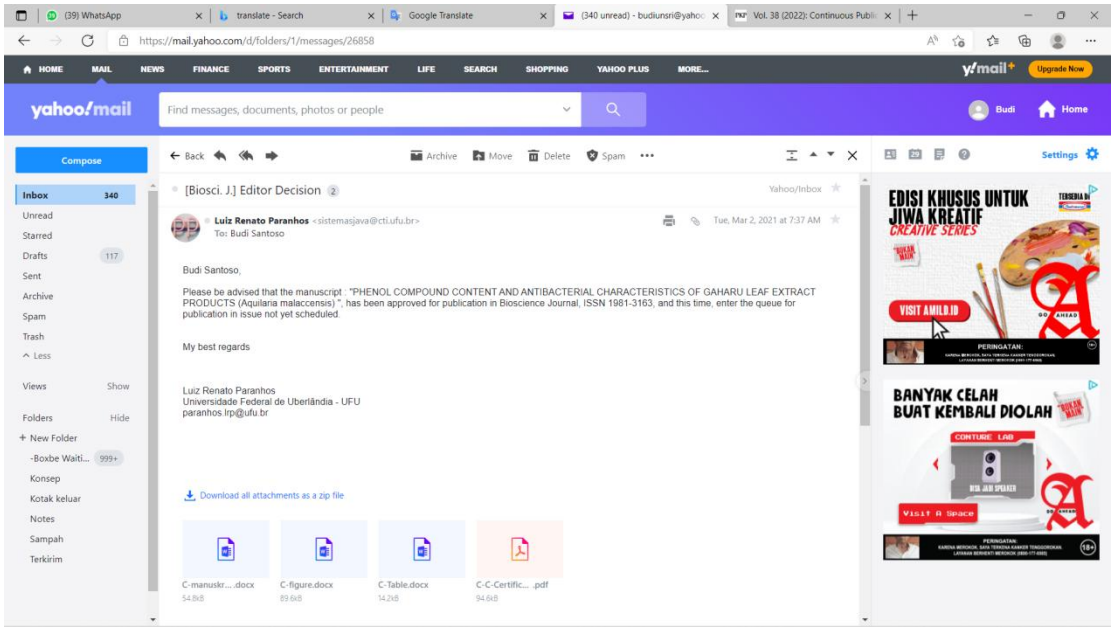
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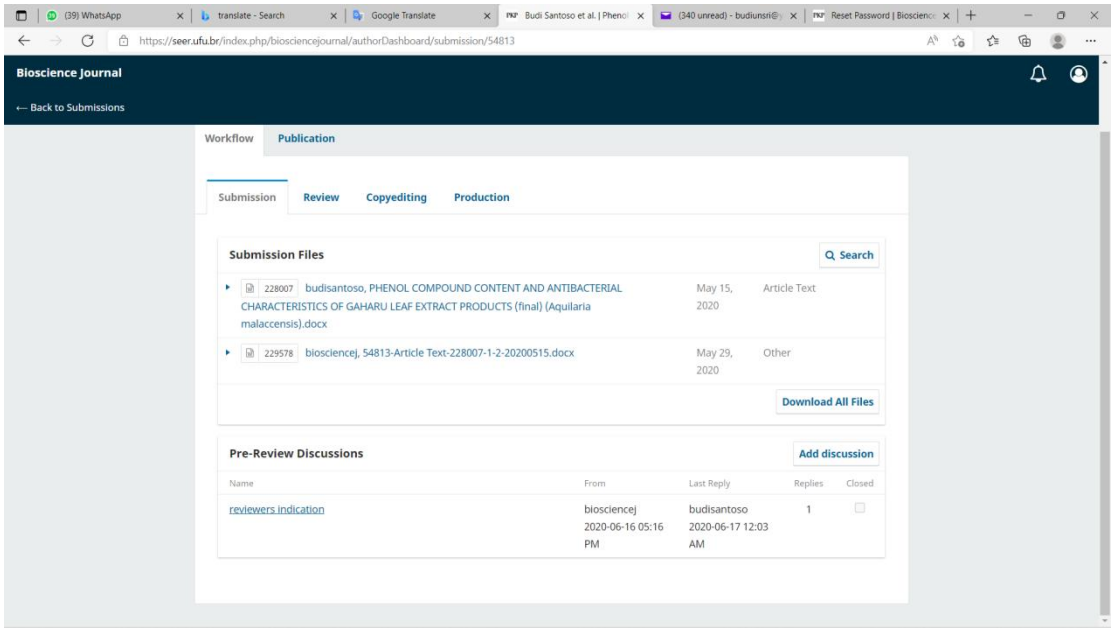
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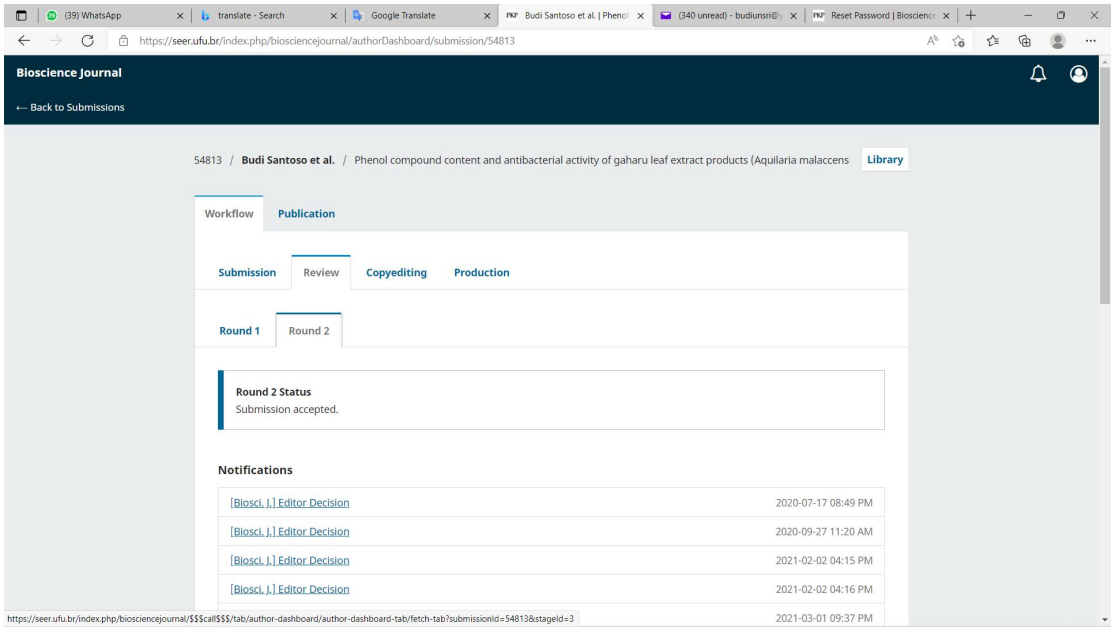
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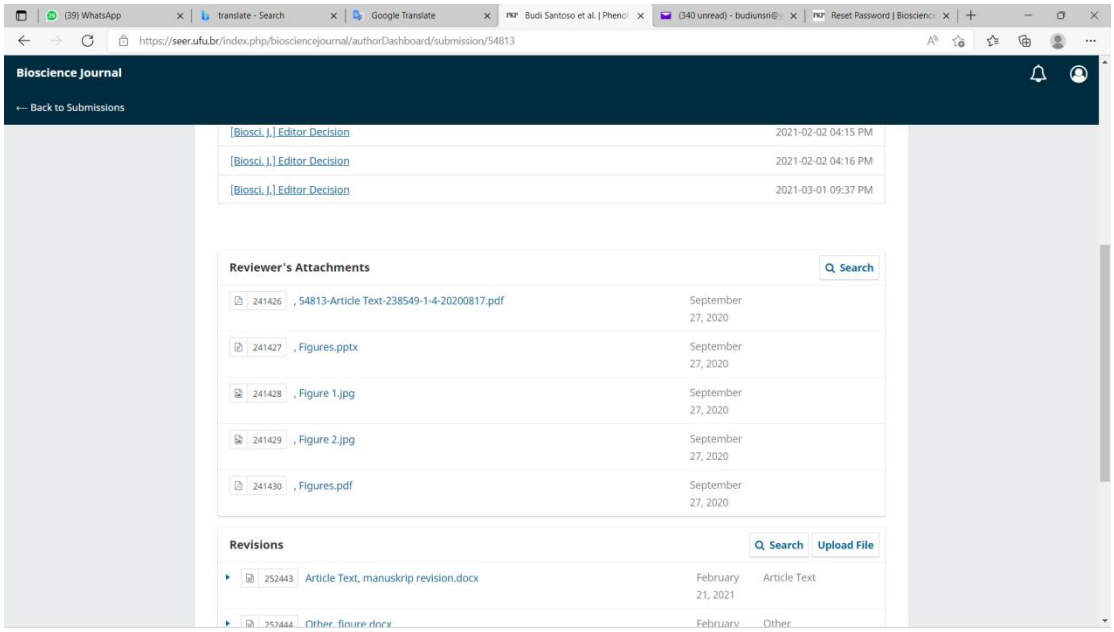
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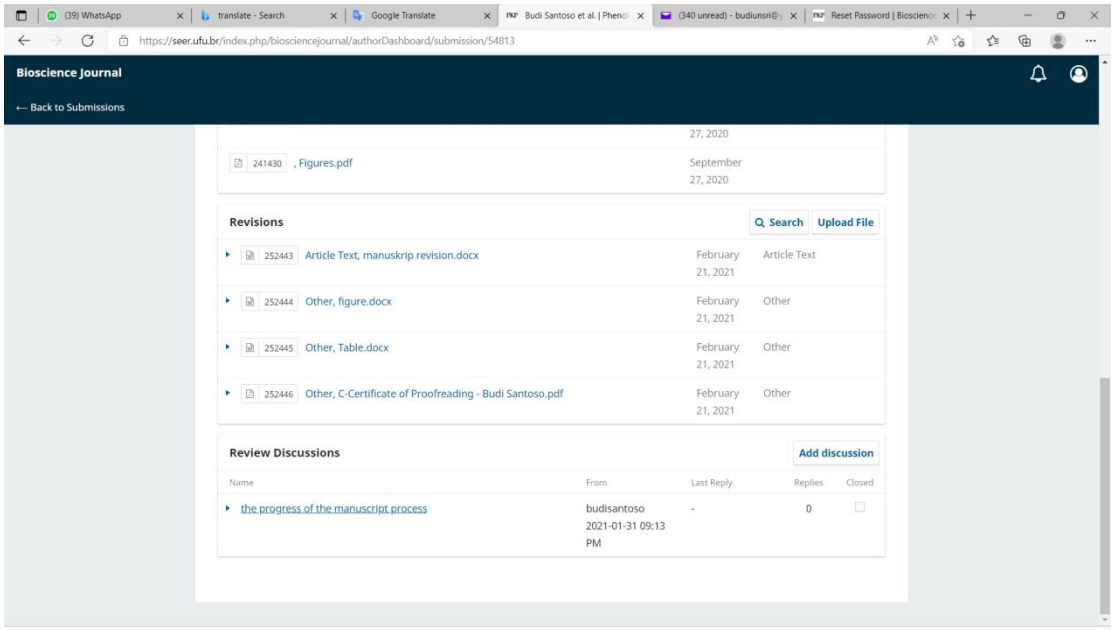
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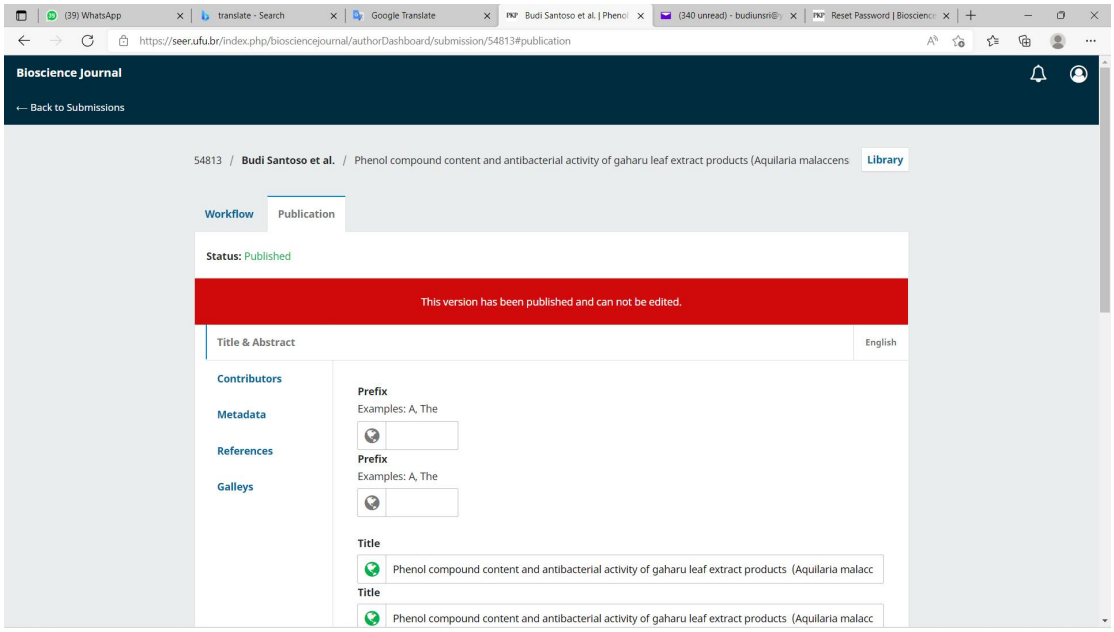
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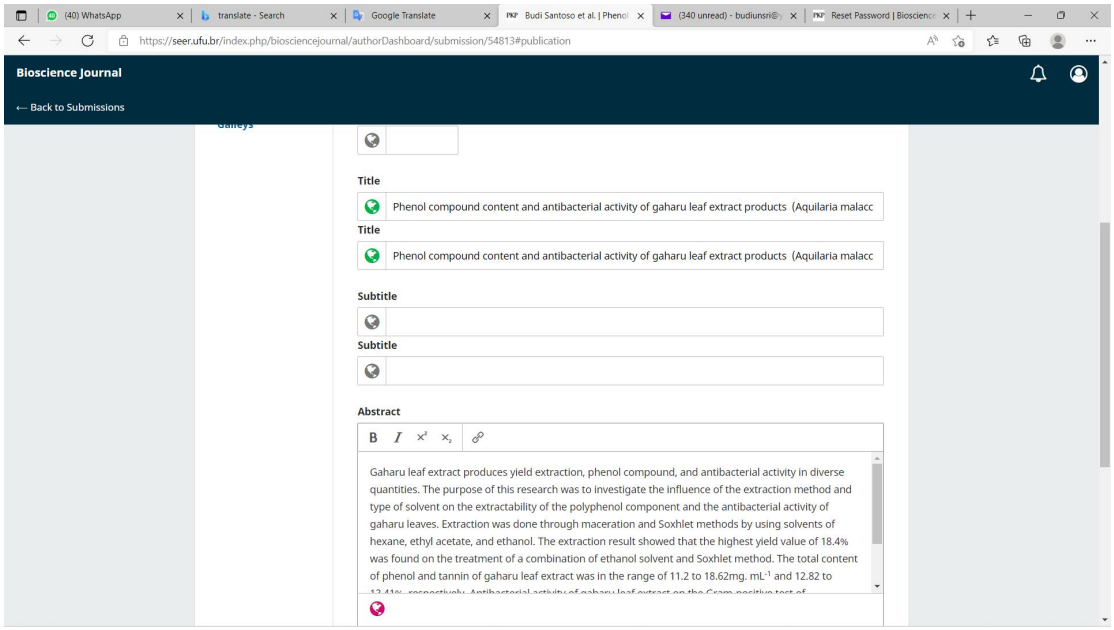
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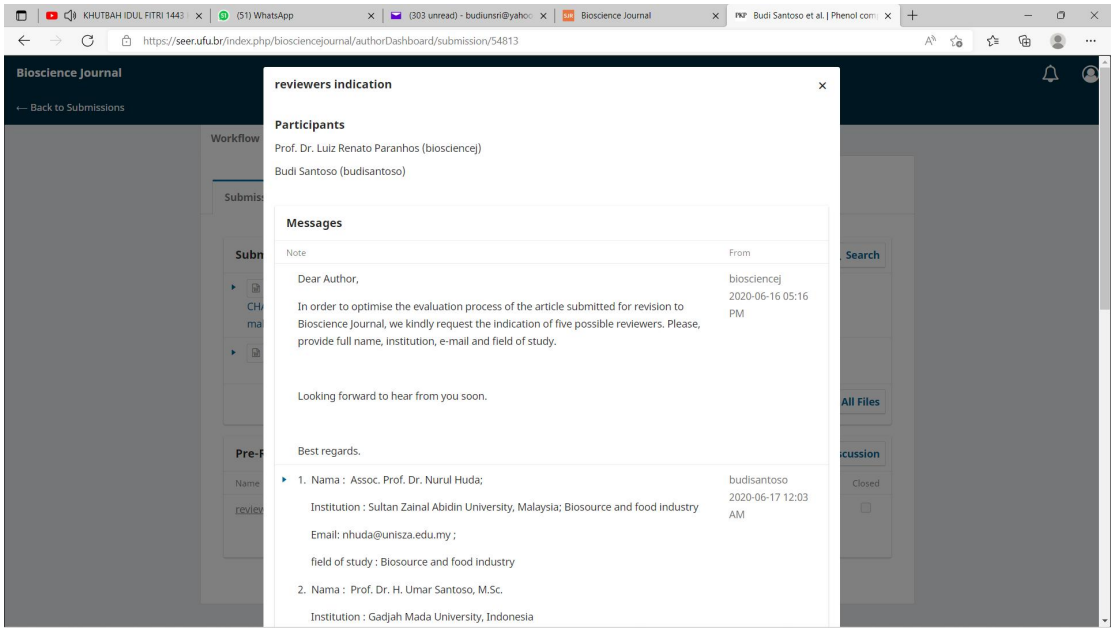
Gaharu leaf extract produces yield extraction, phenol compound, and antibacterial activity in diverse quantities. The purpose of this research was to investigate the influence of the extraction method and type of solvent on the extractability of the polyphenol component and the antibacterial activity of gaharu leaves. Extraction was done through maceration and Soxhlet methods by using solvents of hexane, ethyl acetate, and ethanol. The extraction result showed that the highest yield value of 18.4% was found on the treatment of a combination of ethanol solvent and Soxhlet method. The total content of phenol and tannin of gaharu leaf extract was in the range of 11.2 to 18.62mg. mL-1 and 12.82 to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Email: nhuda@unisza.edu.my ;
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2. Nama : Prof. Dr. H. Umar Santoso, M.Sc.
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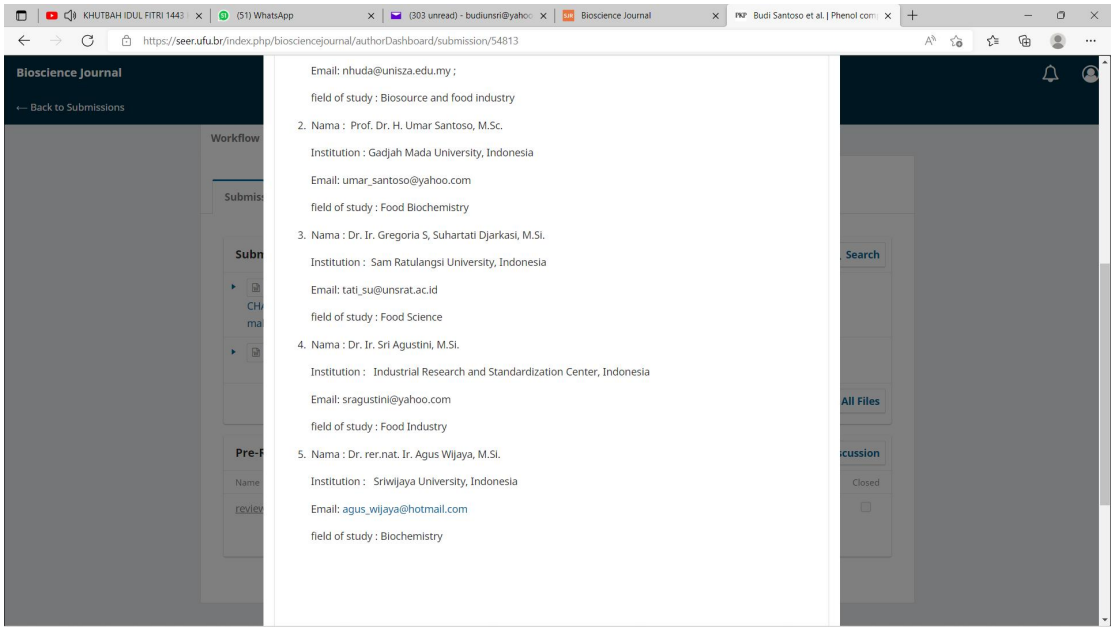

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field of study : Food Biochemistry

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field of study : Food Science

4. Nama : Dr. Ir. Sri Agustini, M.Si.

Institution : Industrial Research and Standardization Center, Indonesia

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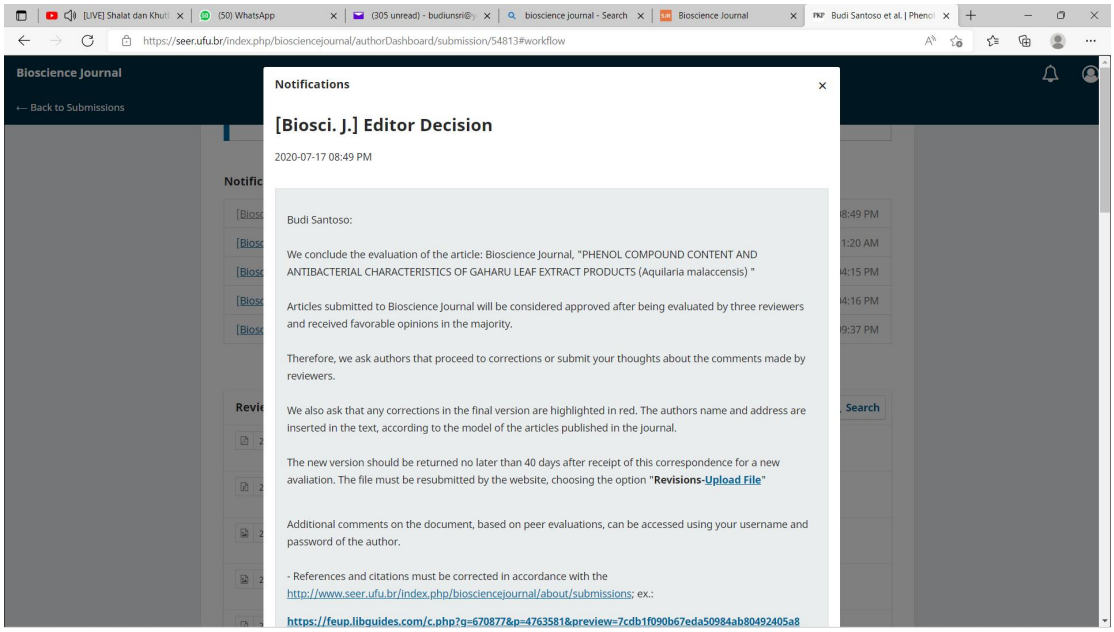

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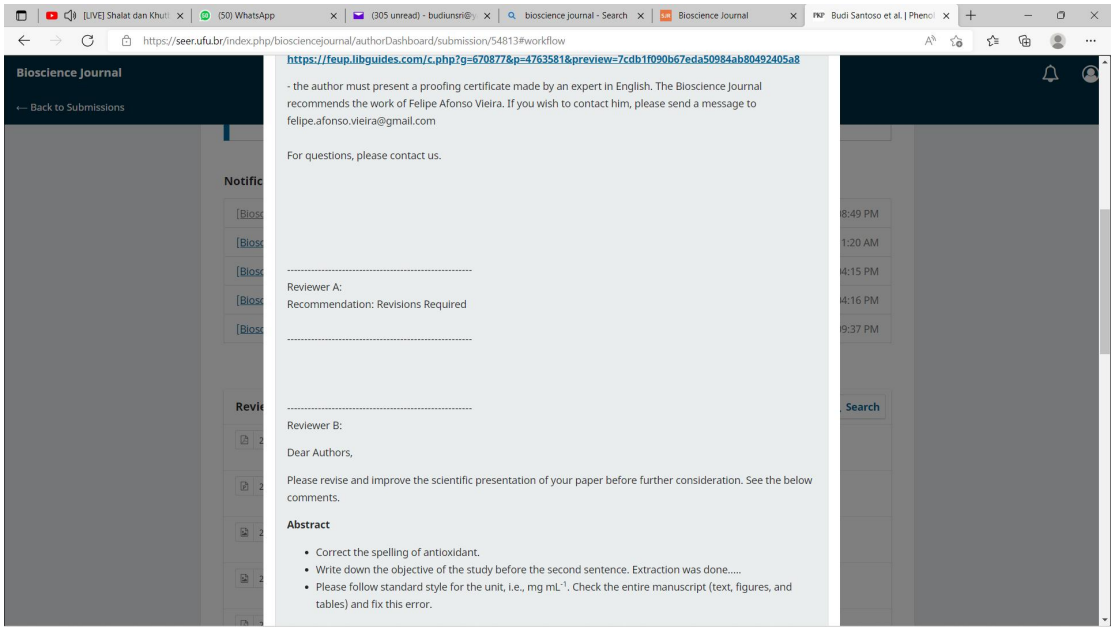
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• Write down the objective of the study before the second sentence. Extraction was done.....
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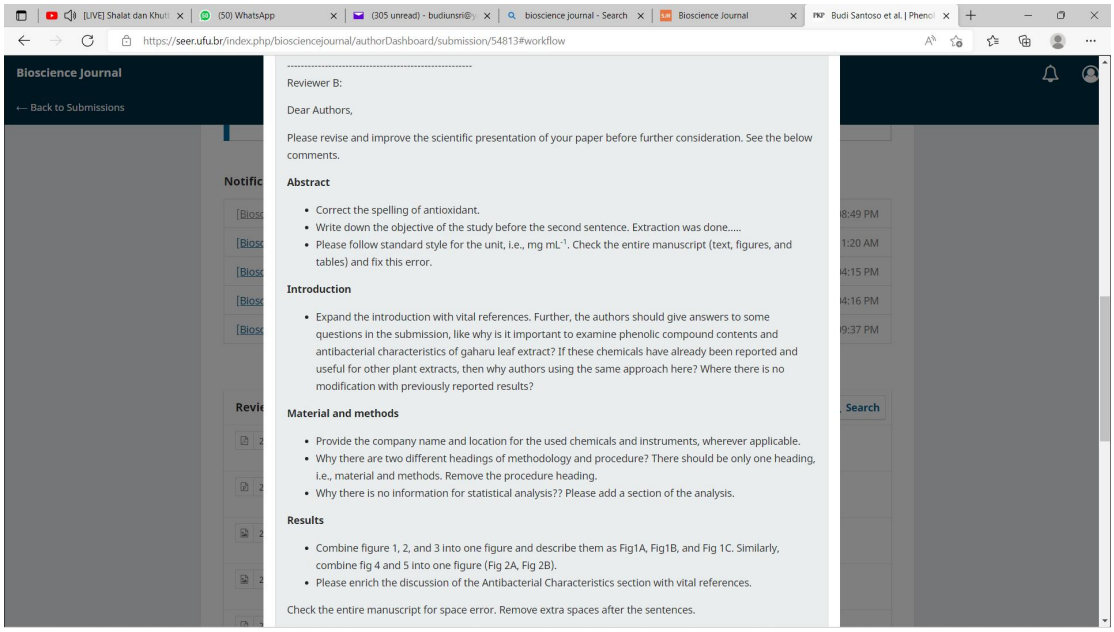
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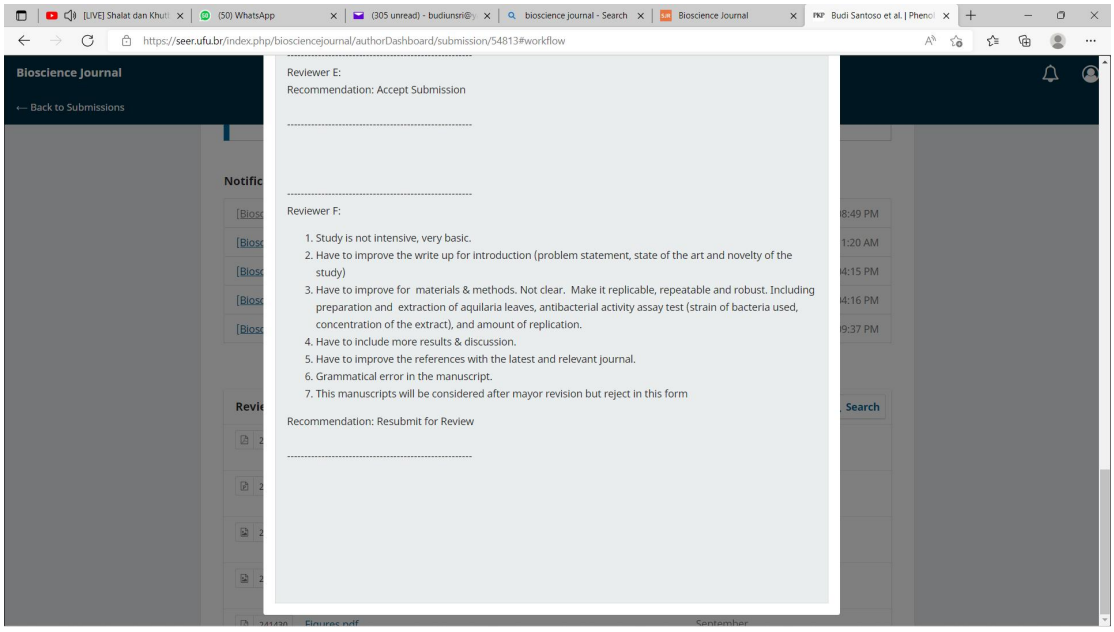
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3. Have to improve for materials & methods. Not clear. Make it replicable, repeatable and robust. Including preparation and extraction of aquilaria leaves, antibacterial activity assay test (strain of bacteria used, concentration of the extract), and amount of replication.
4. Have to include more results & discussion.
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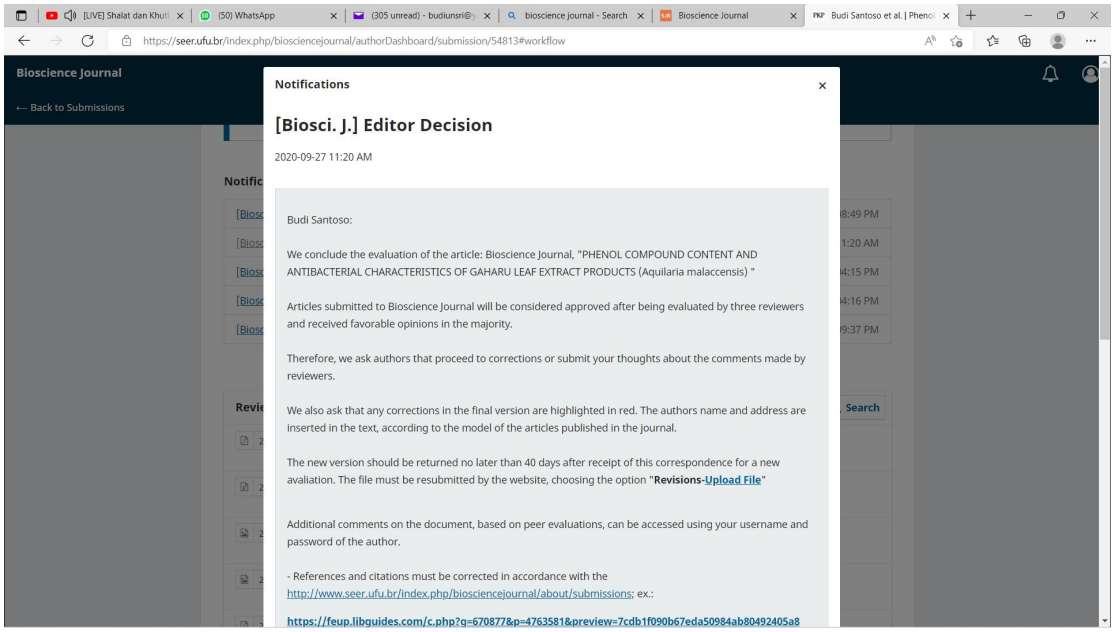
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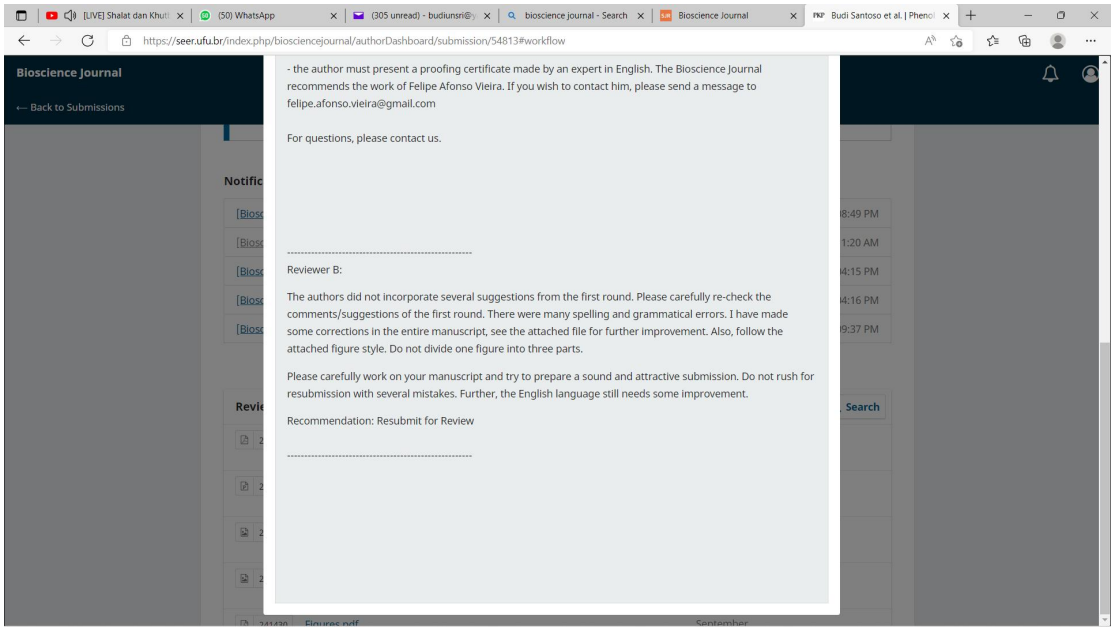
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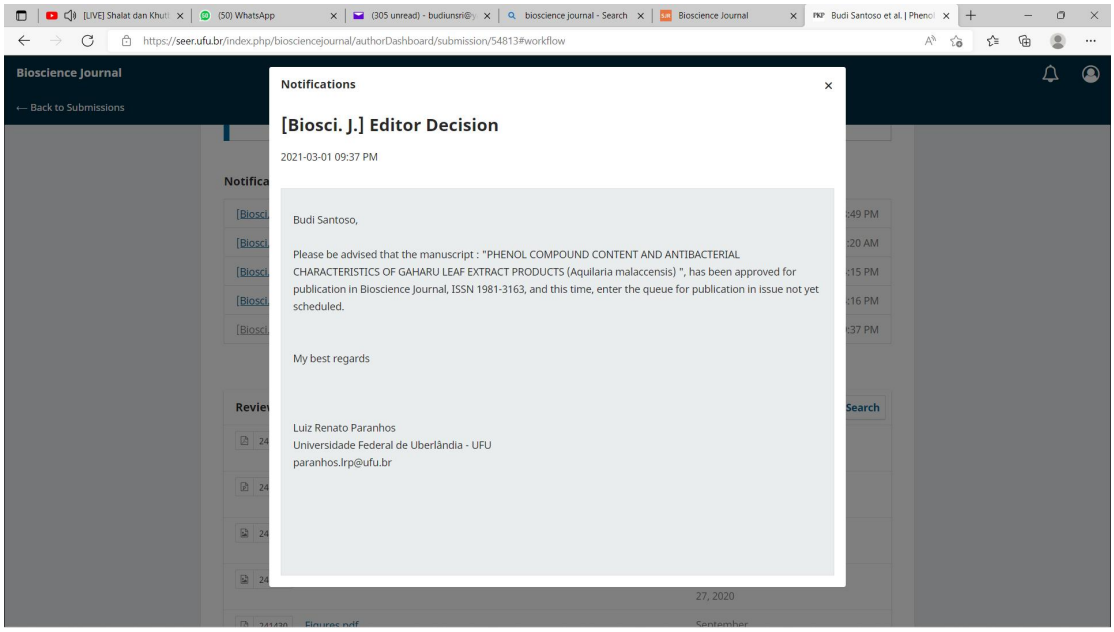
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My best regards
Luiz Renato Paranhos
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PHENOL COMPOUND CONTENT AND ANTIBACTERIAL CHARACTERISTICS OF GAHARU LEAF EXTRACT PRODUCTS (*Aquilaria malaccensis*)

ABSTRACT

Gaharu leaf extract produces threshold, antioxydant compound and antibacterial characteristics in diverse quantity. Extraction was done through maseration and Soxhlet methods by using solvents of hexane, ethyl acetate and ethanol. The extraction result showed that the highest threshold value of 18.4% was found on treatment combination of ethanol solvent and Soxhlet method. Total content of phenol and tannin of gaharu leaf extract was in the range of 11.2 to 18.62 mg/ml and 12.82 to 13.41%, respectively. Antibacterial characteristics of gaharu leaf extract on Gram-positive test of *Staphylococcus aureus* was higher than that of Gram-negative test of *Escherichia coli* having value of zone of inhibition in the range of 5.33 to 6.33 mm and 4.00 to 5.00 mm, respectively. Gaharu leaf extracted with ethanol solvent using Soxhlet method had antioxidant and antibacterial compounds.

Key words: Antibacterial, phenol, leaf extract of *Aquilaria malaccensis*

INTRODUCTION

The leaf of gaharu (*Aquilaria malaccensis*) plant has the functional compound. According to Pranakhon *et al.* (2011), gaharu leaf contains antioxidant compounds which consisted of phenol, terpenoid and flavonoid. These compounds are classified as polyphenol compound group. In addition to these compounds, Kamonwannasit *et al.* (2013) stated that gaharu leaf also contains tannin, saponin and *cardiac glycosides* compounds, but has no alkaloid. Tannin is a stringent compound having bitter taste due to its polyphenol cluster that capable to bind and to precipitate protein. Tannin is water- soluble phenol compound and has molecular weight in the range of 500 to 300 Da.

Polyphenol compound contains hydroxyl cluster which is easily soluble within polar solvent. Different number and position of hydroxyl clusters make this compound has wide spectrum in term of solubility characteristics within

solvent having different polarity levels. Therefore, extraction by using different solvents will also produce different polyphenol compounds.

The extraction process of a substance is not only affected by type and concentration of solvent, but also by substance size, extraction period and temperature as well as extraction method. Extraction methods of Soxhlet and maseration have respective advantages and disadvantages in producing extracted substances. Therefore, the research related to extraction of substance by using these two methods is important to be implemented.

METHODOLOGY

Materials

The tested product is leaf of gaharu plant (*Aquilaria malaccensis*) obtained from Bangka Tengah District, Bangka Belitung Province. Chemical substances used in this study were *Folin Ciocalteau* 50%, alcohol, galate acid, aquadest, gelatine, indogocarmine, kaoline powder, *Nutrien Broth*, *Plate Count Agar*, and extraction solvents of hexane, ethyl acetate and ethanol. Gram-negative bacterium of *Escherichia coli* and Gram-positive bacterium of *Staphylococcus aureus* are used in this study.

Equipments

Soxhlet extractor, Erlenmeyer flask of 1 L, *Shaker waterbath, Rotaporator*, spectrophotometer, autoclave, incubator, petri dish, Eppendorf micro pipete, ose needle, and *Mixer Vortex*.

Exprimental Design

The exprimental design used in this study was Randomized Block Design consisting of two treatment factors of extraction methods (A_1 = Soxhlet and A_2 = maseration) and solvent types (B_1 = hexane , B_2 = ethyl acetate and B_3 = ethanol). The observed parameters were threshold (Lubis, 2008), total phenol (Pourmorad

et al., 2006), tannin concentration (Sudarmadji *et al*., 2007), antimicrobia test (Miller and Shah, 2000), water content (Sudarmadji *et al.,* 2007) and ash content (Sudarmadji *et al*., 2007).

Procedure

1. Soxhlet Extraction

Sample of gaharu leaf powder is weighed with magnitude of 40 g, wrapped with sieving paper and then put into Soxhlet tube. Soxhlet flask is filled with solvent having volume of 250 mL. Soxhlet unit is set up and equipped with reverse cooler, heated at boiling point temperature of solvent, let the circulation proceeded until solvent color is clear. The produced solution is subsequently rotaevaporated by using *vacuum rotary evaporator* at proper pressure and temperature of solvent until dry extract was produced.

2. Maseration Extraction

Sample of gaharu leaf powder is weighed with magnitude of 40 g, put into vessel (maserator) and added with solvent according to treatments having volume of 250mL. Solution is subsequently maserated for 3 x 24 hours at room temperature and filtrated by using sieve paper. The produced filtrate is evaporated by using *vacuum rotary evaporator* until viscous extract is obtained. This viscous extract was subsequently dried within oven for 48 hours until dry extract is produced.

RESULTS AND DISCUSSION

Extraction Threshold

Results of extraction threshold from two extraction methods showed that the higher the solvent polarity level, the higher the threshold magnitude. The highest threshold value of gaharu leaf extract was 18.4% (A_1B_3 treatment) and the lowest threshold value of gaharu leaf extract was 0.73% (A_2B_1 treatment) such as shown in Figure 1.

Analysis of variance results showed that extraction method, solvent type and their interactions had significant effect on extraction threshold. Results of HSD test was shown in Table 1.

Table 1. HSD test results related to the effect of extraction method, solvent type and their interactions on threshold of gaharu leaf extract.

Extraction method	Threshold (%)
A2 (Maseration)	3.50a
A1 (Soxhlet)	8.50b
Sovent type	Threshold (%)
B1(hexana)	1.41a
B2 (ethyl acetate)	3.40b
B3 (ethanol)	13.20c
Interaction	Threshold (%)
A2B1	0.73a
A2B2	1.78ab
A1B1	2.09b
A1B2	5.02c
A2B3	8.00d
A1B3	18.4e

Remarks: Numbers followed by the same letter in the same column are not significantly different (at α =5%).

Results of HSD test (Table 1) showed that soxhlet extraction method produced higher threshold value than that of maseration extraction method. This was due to the fact that soxhlet method used heating treatment that had effect on increasing kinetic energy of solvent. The higher the kinetic energy, the easier the solvent diffusion into cell tissues of gaharu leaf resulting in higher quantity of extract. Daud *et al.* (2011) stated that Soxhlet extraction method had produced higher threshold value of guava leaf than that of maseration method with magnitudes of 25.00% and 18.47%, respectively.

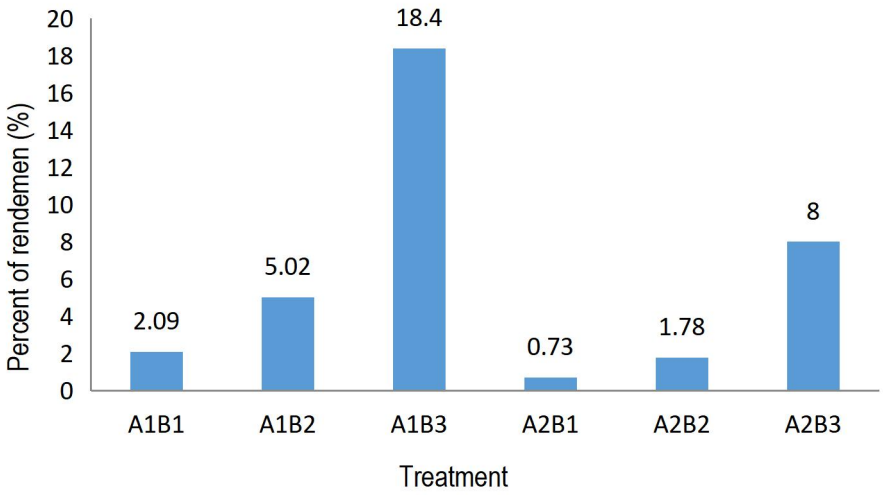

Figure 1. Extraction threshold of gaharu leaf using extraction method of soxhlet (A1) and maseration (A2) with solvents having different polarity levels (B1= hexane, B2 = ethyl acetate and B3 = ethanol).

Different threshold values of gaharu leaf extract using different solvents were due to differences of polarity level of respective solvents. The higher the solvent polarity value, the higher the extraction threshold produced such as shown by HSD test results (Table 1). High solubility is related to polarity of solvent and polarity of the extracted substance (*like dissolves like*). Polarity of solvent is indicated by dipole moment, dielectric constant and solubility in water. Ethanol has higher polarity index than that of ethyl acetate and hexane so that it had produced higher threshold than that of ethyl acetate and hexane (Azizahwati *et al*., 2017).

Results of HSD test (Table 1) showed that treatment interaction of soxhlet extraction method and ethanol solvent had produced the highest threshold. Soxhlet extraction method uses higher temperature that cause lower viscosity of solvent and higher extract solubility which in turn affect the produced threshold. Ethanol has the highest polarity index than the other solvents; the higher the solvent polarity, the higher the produced extract. Hatam *et al*. (2013) had described that pineapple skin extraction by using soxhlet method and ethanol

solvent produced higher total phenol than that of maseration method using the same solvent.

Total Phenol

The highest total phenol with magnitude of 18.62mg/mL was found on treatment of Soxhlet extraction method using ethyl acetate solvent, whereas the lowest one with magnitude of 9.62mg/mL was found on treatment of maseration extraction method using hexane solvent. Average values of total phenol from gaharu leaf extract was shown in Figure 2.

Results of variance analysis showed that solvent types had significant effect on the produced total phenol, whereas the interaction of extraction method and solvent types had no signifcant effect on the produced total phenol. Results of HSD test of solvent types on average values of total phenol was presented in Table 2.

Table 2. Results of HSD test for the effect of solvent types on gaharu leaf threshold.

Solvent type	Threshold (%)
B_1 (hexane)	10.41a
B_3 (ethanol)	12.72a
B_2 (ethyl acetate)	17.92b

Remarks: Numbers followed by the same letters in the same column are not significantly different (at α =5%).

Ethyl acetate solvent produced the highest total phenol than that of other solvents. This is due to the fact that phenol compound has semipolar characteristic so that it will more solube within semipolar solvent. Semipolar solvents such as ethyl acetate can extract phenol, terpenoid, alkaloid, aglycon and aglycide compounds (Al-Ash'ary *et al.,* 2010). This is in accordance to finding by Hagerman (2002) which showed that phenol compound generally is difficult to precipitate in cold water. Pambayun *et al.* (2007) had added that the highest total phenolate was obtained from gambier extraction process with Soxhlet method by using ethyl acetate.

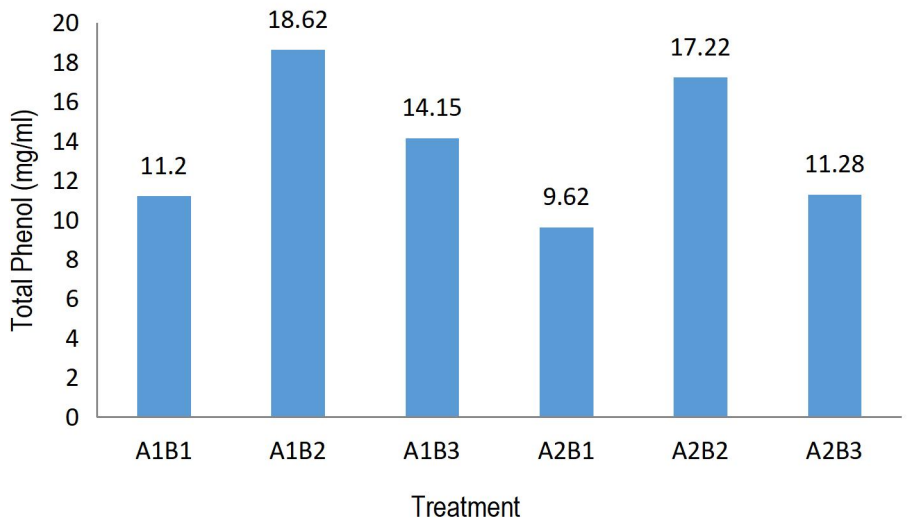

Figure 2. Total phenol of gaharu leaf produced by using extraction methods of soxhlet (A_1) and maseration (A_2) with solvents having different polarity levels (B_1 = hexane, B_2 = ethyl acetate and B_3 = ethanol).

Tannin

The highest tannin concentration of gaharu leaf extract with magnitude of 13.41% was found on treatment of Soxhlet method and ethyl acetate solvent, whereas the lowest tannin concentration with magnitude of 12.82% was found on treatment of maseration method and n-hexane solvent. These results were similar to the finding by Ginting *et al.* (2015) which stated that gaharu leaf tea had bitter taste and contain tannin concentration of 0.2571%. Hadi *et al*. (2011) had added that gaharu leaf extract contains terpenoid, tannin and flavonoid. Average value of tannin concentration was shown in Figure 3.

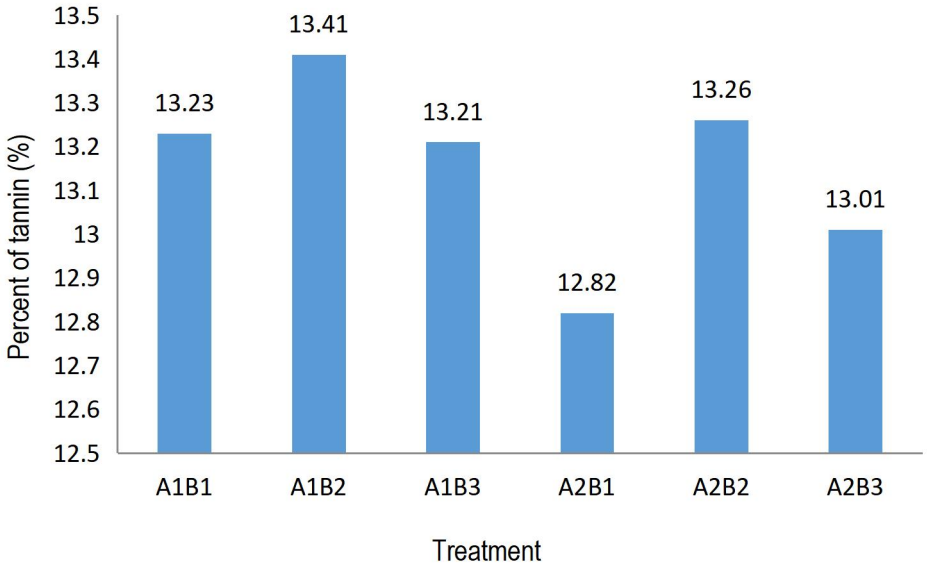

Figure 3. Tannin concentration of gaharu leaf produced by using extraction methods of Soxhlet (A_1) and maseration (A_2) with solvents having different polarity levels (B_1 = hexane, B_2 = ethyl acetate and B_3 = ethanol).

Antibacterial Characteristics

Antibacterial characteristics of gaharu leaf extract obtained by using Soxhlet and maseration methods as well as different solvents was explained by zone of inhibition toward the tested bacteria. Average value of zone of inhibition toward *Escherichia coli* bacterium was shown in Figure 4.

Zone of inhibition of gaharu leaf extract toward bacterial test of *Escherichia coli* (Gram-negative) with magnitude of 4.67mm was lower than that of bacterial test of *Staphylococcus aureus* (Gram-positive) with magnitude of 6.33mm. Capability of gaharu leaf extract as antibacterial agent for Gram-positive and Gram-negative bacteria is due to phenol and tannin compounds within gaharu leaf. However, zone of inhibition value of gaharu leaf extract toward Gram-positive bacterium was higher than that of Gram-negative bacterium. This results was strengthened by Kamonwannasit *et al.* (2013) finding which stated that gaharu

leaf extract was capable to impede *Staphylococcus epidermidis* bacterium with zone of inhibition value of 12 mm.

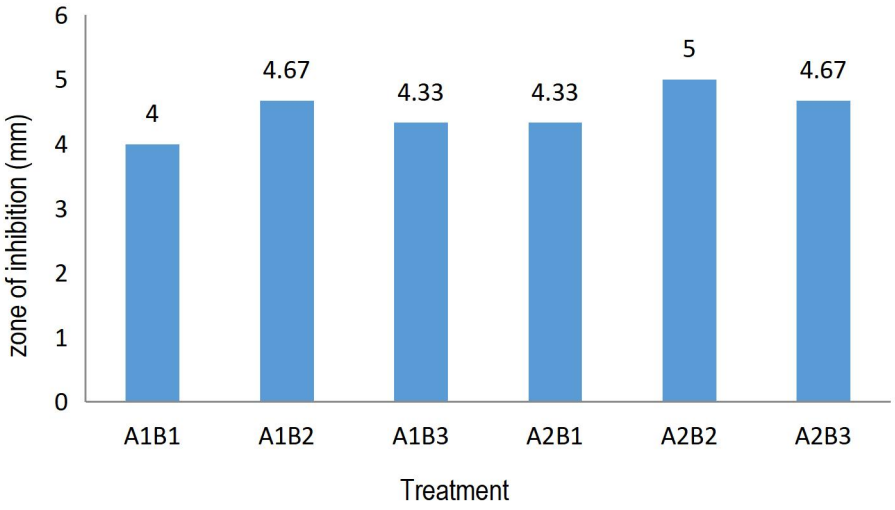

Figure 4. The RCD (mm) value of bacterial test of *Escherichia coli* on gaharu leaf extract.

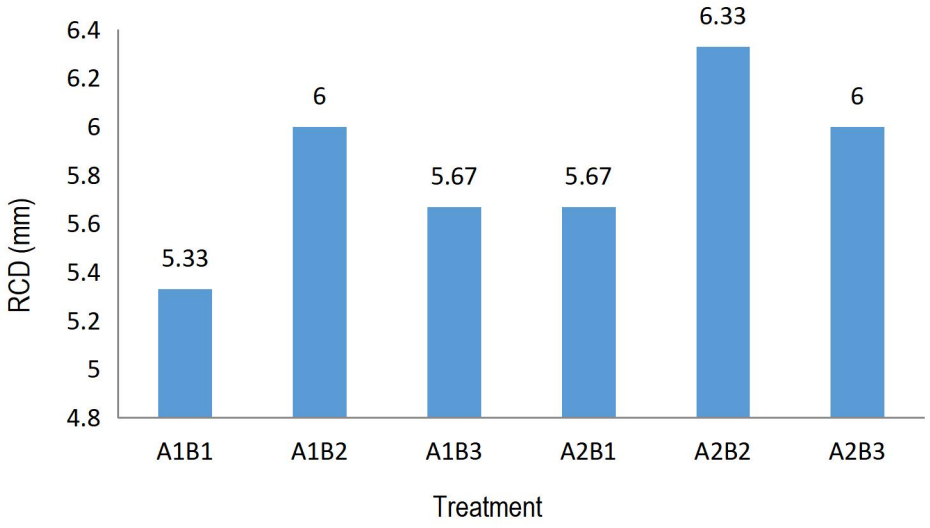

Figure 5. The RCD (mm) value of bacterial test of *Staphylococcus aureus* on gaharu leaf extract

There are two mechanisms related to bacteria growth obstruction by OH^- ions, i.e. OH^- ions bind the bacterium cell wall that contain high concenration of peptidoglycan such as found in Gram-positive bacterium. Peptidoglycan found in Gram-positive bacterium is relatively thicker with magnitude of about 40 nm and located at surface, whereas Gram-negative bacterium has thinner peptidoglycan and located at inside. Based on position of peptidoglycan, peptidoglycan position of Gram-positive bacterium is more accessible by OH^- ions than that of Gram-negative bacterium.

CONCLUSION

1. Soxhlet extraction method had produced higher threshold of gaharu leaf extract than that of maseration method.
2. The highest total phenol concentration was found on extraction method using ethyl acetate solvent.
3. Antibacterial characteristics of gaharu leaf extract was higher toward Gram-positive bacterium than that of Gram-negative bacterium.

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ABSTRACT

Gaharu leaf extract produces threshold, antioxydant compound and antibacterial characteristics in diverse quantity. Extraction was done through maseration and Soxhlet methods by using solvents of hexane, ethyl acetate and ethanol. The extraction result showed that the highest threshold value of 18.4% was found on treatment combination of ethanol solvent and Soxhlet method. Total content of phenol and tannin of gaharu leaf extract was in the range of 11.2 to 18.62 mg mL^{-1} and 12.82 to 13.41%, respectively. Antibacterial characteristics of gaharu leaf extract on Gram-positive test of *Staphylococcus aureus* was higher than that of Gram-negative test of *Escherichia coli* having value of zone of inhibition in the range of 5.33 to 6.33 mm and 4.00 to 5.00 mm, respectively. Gaharu leaf extracted with ethanol solvent using Soxhlet method had antioxidant and antibacterial compounds.

Key words:, phenol, leaf extract, , Soxhlet method

According to the journal's rules, it is necessary to include an abstract in Portuguese.

INTRODUCTION

The leaf of gaharu (*Aquilaria malaccensis*) plant has the functional compound. According to Pranakhon *et al.* (2011), gaharu leaf contains antioxidant compounds which consisted of phenol, terpenoid and flavonoid. These compounds are classified as polyphenol compound group. In addition to these compounds, Kamonwannasit *et al.* (2013) stated that gaharu leaf also contains tannin, saponin and *cardiac glycosides* compounds, but has no alkaloid. Tannin is a stringent compound having bitter taste due to its polyphenol cluster that capable to bind and to precipitate protein. Tannin is water- soluble phenol compound and has molecular weight in the range of 500 to 300 Da.

Polyphenol compound contains hydroxyl cluster which is easily soluble within polar solvent. Different number and position of hydroxyl clusters make this compound has wide spectrum in term of solubility characteristics within

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solvent having different polarity levels. Therefore, extraction by using different solvents will also produce different polyphenol compounds.

The extraction process of a substance is not only affected by type and concentration of solvent, but also by substance size, extraction period and temperature as well as extraction method. Extraction methods of Soxhlet and maseration have respective advantages and disadvantages in producing extracted substances. Therefore, the research related to extraction of substance by using these two methods is important to be implemented.

It includes the purpose of the work explicitly here.

METHODOLOGY

Materials

The tested product is leaf of gaharu plant (*Aquilaria malaccensis*) obtained from Bangka Tengah District, Bangka Belitung Province. Chemical substances used in this study were *Folin Ciocalteau* 50%, alcohol, galate acid, aquadest, gelatine, indogocarmine, kaoline powder, *Nutrien Broth*, *Plate Count Agar*, and extraction solvents of hexane, ethyl acetate and ethanol. Gram-negative bacterium of *Escherichia coli* and Gram-positive bacterium of *Staphylococcus aureus* are used in this study.

Equipments

Soxhlet extractor, Erlenmeyer flask of 1 L, *Shaker waterbath, Rotaporator*, spectrophotometer, autoclave, incubator, petri dish, Eppendorf micro pipete, ose needle, and *Mixer Vortex*.

Exprimental Design

The exprimental design used in this study was Randomized Block Design consisting of two treatment factors of extraction methods (A_1 = Soxhlet and A_2 = maseration) and solvent types (B_1 = hexane , B_2 = ethyl acetate and B_3 = ethanol).

It is necessary to present which statistical tests were performed and which software was used.

The observed parameters were threshold (Lubis, 2008), total phenol (Pourmorad *et al.,* 2006), tannin concentration (Sudarmadji *et al*., 2007), antimicrobia test (Miller and Shah, 2000), water content (Sudarmadji *et al.,* 2007) and ash content (Sudarmadji *et al*., 2007).

Procedure

1. Soxhlet Extraction

Sample of gaharu leaf powder were weighed with magnitude of 40 g, wrapped with sieving paper and then put into Soxhlet tube. Soxhlet flask were filled with solvent having volume of 250 mL. Soxhlet unit were set up and equipped with reverse cooler, heated at boiling point temperature of solvent, let the circulation proceeded until solvent color is clear. The produced solution is subsequently rotaevaporated by using *vacuum rotary evaporator* at proper pressure and temperature of solvent until dry extract was produced.

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Sample of gaharu leaf powder were weighed with magnitude of 40 g, put into vessel (maserator) and added with solvent according to treatments having volume of 250 mL. Solution were subsequently maserated for 3 x 24 hours at room temperature and filtrated by using sieve paper. The produced filtrate were evaporated by using *vacuum rotary evaporator* until viscous extract is obtained. This viscous extract was subsequently dried within oven for 48 hours until dry extract is produced.

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RESULTS AND DISCUSSION

Extraction Threshold

Results of extraction threshold from two extraction methods showed that the higher the solvent polarity level, the higher the threshold magnitude. The highest threshold value of gaharu leaf extract was 18.4% (A_1B_3 treatment) and the

lowest threshold value of gaharu leaf extract was 0.73% (A_2B_1 treatment) such as shown in Figure 1.

Analysis of variance results showed that extraction method, solvent type and their interactions had significant effect on extraction threshold. Results of HSD test was shown in Table 1.

Table 1. HSD test results related to the effect of extraction method, solvent type and their interactions on threshold of gaharu leaf extract.

Extraction method	Threshold (%)
A2 (Maseration)	3.50a
A1 (Soxhlet)	8.50b
Sovent type	**Threshold (%)**
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B2 (ethyl acetate)	3.40b
B3 (ethanol)	13.20c
Interaction	**Threshold (%)**
A2B1	0.73a
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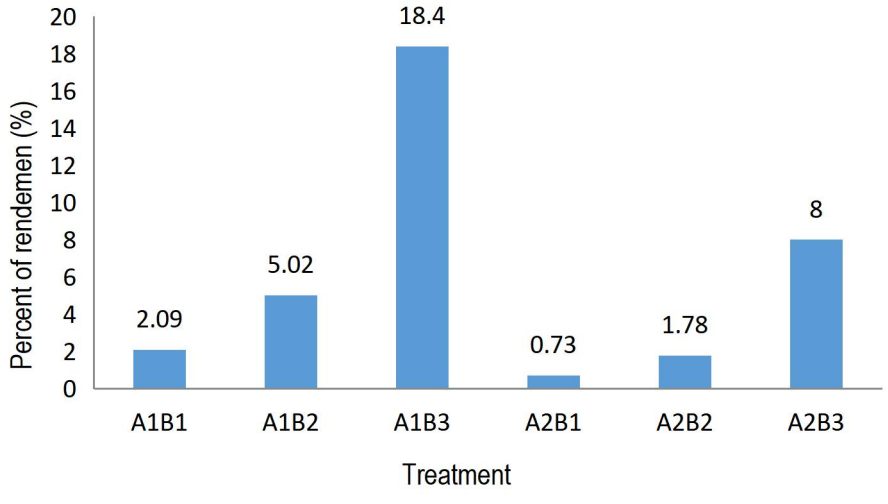

Figure 1. Extraction threshold of gaharu leaf using extraction method of soxhlet (A1) and maseration (A2) with solvents having different polarity levels (B1= hexane, B2 = ethyl acetate and B3 = ethanol).

Different threshold values of gaharu leaf extract using different solvents were due to differences of polarity level of respective solvents. The higher the solvent polarity value, the higher the extraction threshold produced such as shown by HSD test results (Table 1). High solubility is related to polarity of solvent and polarity of the extracted substance (*like dissolves like*). Polarity of solvent is indicated by dipole moment, dielectric constant and solubility in water. Ethanol has higher polarity index than that of ethyl acetate and hexane so that it had produced higher threshold than that of ethyl acetate and hexane (Azizahwati *et al*., 2017).

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Total Phenol

The highest total phenol with magnitude of 18.62 mg mL^{-1} was found on treatment of Soxhlet extraction method using ethyl acetate solvent, whereas the lowest one with magnitude of 9.62 mg mL^{-1} was found on treatment of maseration extraction method using hexane solvent. Average values of total phenol from gaharu leaf extract was shown in Figure 2.

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Ethyl acetate solvent produced the highest total phenol than that of other solvents. This is due to the fact that phenol compound has semipolar characteristic so that it will more solube within semipolar solvent. Semipolar solvents such as ethyl acetate can extract phenol, terpenoid, alkaloid, aglycon and aglycide compounds (Al-Ash'ary *et al.,* 2010). This is in accordance to finding by Hagerman (2002) which showed that phenol compound generally is difficult to precipitate in cold water. Pambayun *et al.* (2007) had added that the highest total phenolate was obtained from gambier extraction process with Soxhlet method by using ethyl acetate.

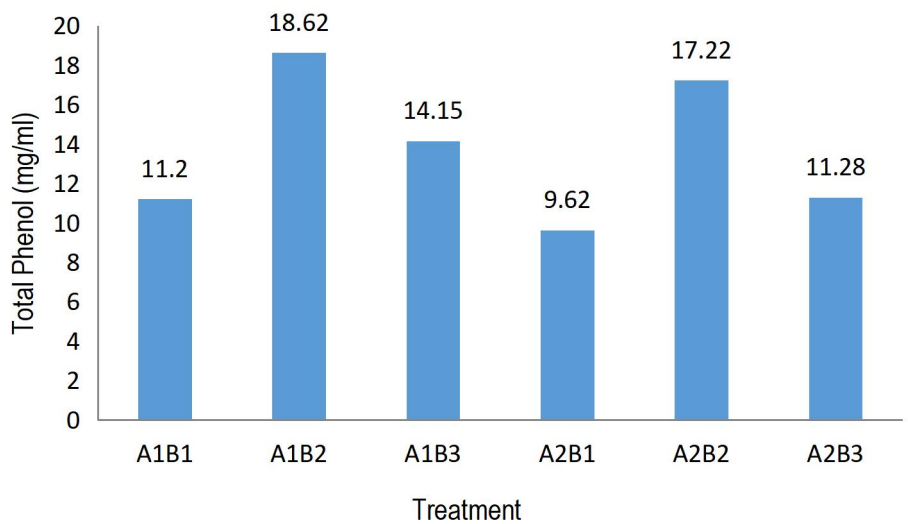

Figure 2. Total phenol of gaharu leaf produced by using extraction methods of soxhlet (A_1) and maseration (A_2) with solvents having different polarity levels (B_1 = hexane, B_2 = ethyl acetate and B_3 = ethanol).

Tannin

The highest tannin concentration of gaharu leaf extract with magnitude of 13.41% was found on treatment of Soxhlet method and ethyl acetate solvent, whereas the lowest tannin concentration with magnitude of 12.82% was found on treatment of maseration method and n-hexane solvent. These results were similar to the finding by Ginting *et al.* (2015) which stated that gaharu leaf tea had bitter taste and contain tannin concentration of 0.2571%. Hadi *et al*. (2011) had added that gaharu leaf extract contains terpenoid, tannin and flavonoid. Average value of tannin concentration was shown in Figure 3.

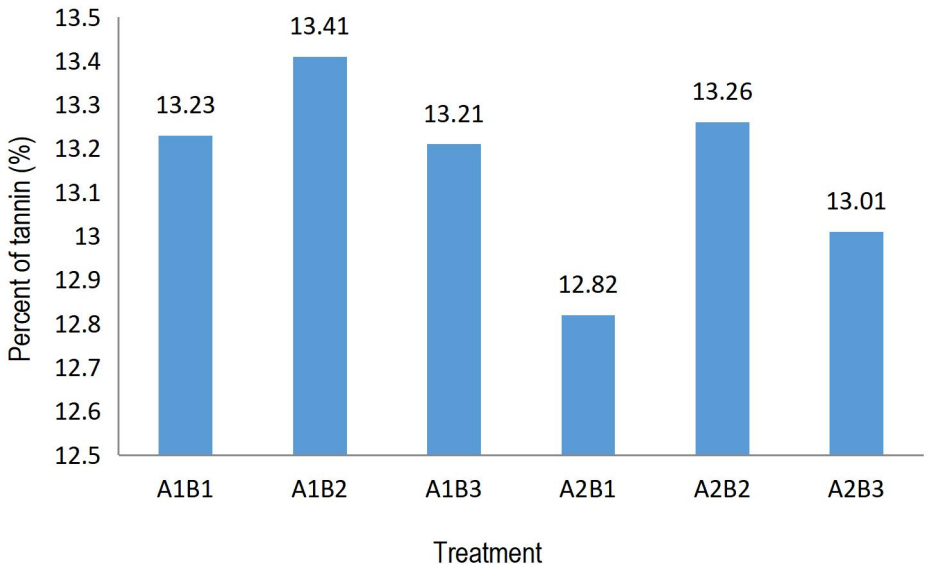

Figure 3. Tannin concentration of gaharu leaf produced by using extraction methods of Soxhlet (A_1) and maseration (A_2) with solvents having different polarity levels (B_1 = hexane, B_2 = ethyl acetate and B_3 = ethanol).

Antibacterial Characteristics

Antibacterial characteristics of gaharu leaf extract obtained by using Soxhlet and maseration methods as well as different solvents was explained by zone of inhibition toward the tested bacteria. Average value of zone of inhibition toward *Escherichia coli* bacterium was shown in Figure 4.

Zone of inhibition of gaharu leaf extract toward bacterial test of *Escherichia coli* (Gram-negative) with magnitude of 4.67 mm was lower than that of bacterial test of *Staphylococcus aureus* (Gram-positive) with magnitude of 6.33 mm. Capability of gaharu leaf extract as antibacterial agent for Gram-positive and Gram-negative bacteria is due to phenol and tannin compounds within gaharu leaf. However, zone of inhibition value of gaharu leaf extract toward Gram-positive bacterium was higher than that of Gram-negative bacterium. This results was strengthened by Kamonwannasit *et al.* (2013) finding which stated that gaharu

leaf extract was capable to impede *Staphylococcus epidermidis* bacterium with zone of inhibition value of 12 mm.

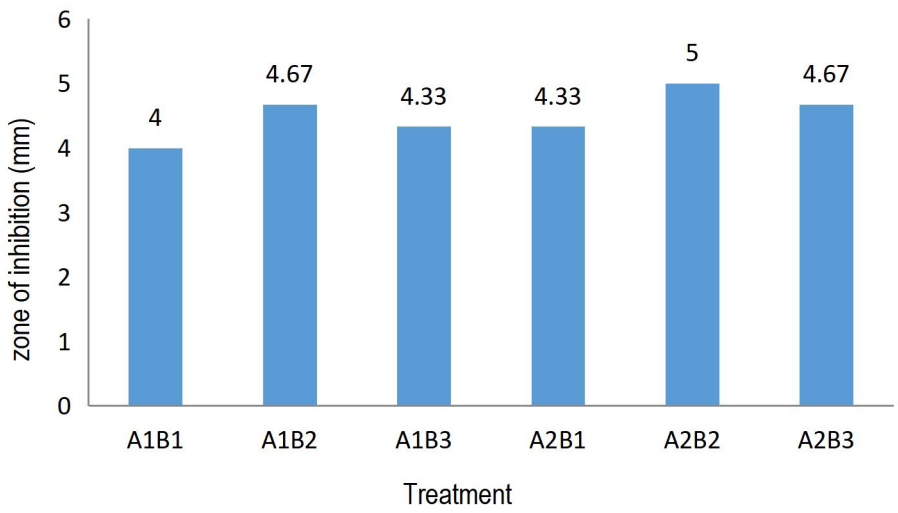

Figure 4. The RCD (mm) value of bacterial test of *Escherichia coli* on gaharu leaf extract.

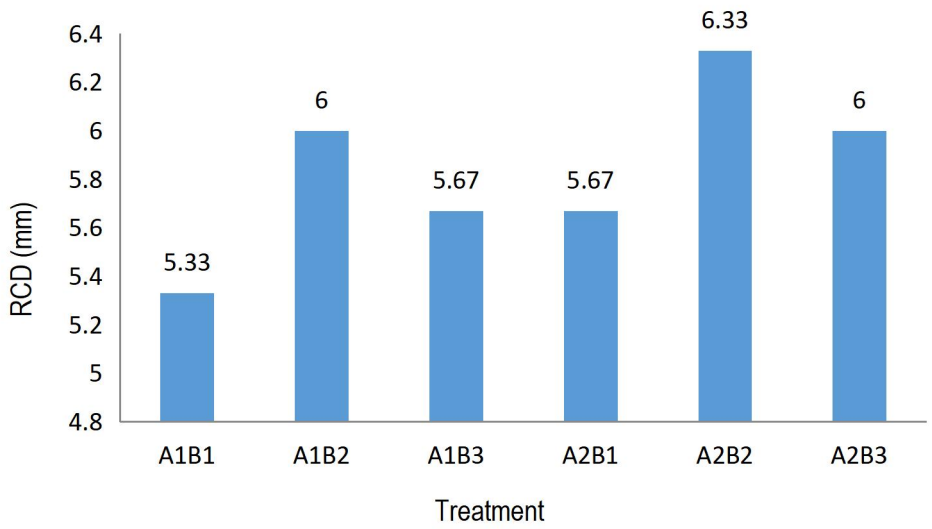

Figure 5. The RCD (mm) value of bacterial test of *Staphylococcus aureus* on gaharu leaf extract

There are two mechanisms related to bacteria growth obstruction by OH^- ions, i.e. OH^- ions bind the bacterium cell wall that contain high concenration of peptidoglycan such as found in Gram-positive bacterium. Peptidoglycan found in Gram-positive bacterium is relatively thicker with magnitude of about 40 nm and located at surface, whereas Gram-negative bacterium has thinner peptidoglycan and located at inside. Based on position of peptidoglycan, peptidoglycan position of Gram-positive bacterium is more accessible by OH^- ions than that of Gram-negative bacterium.

CONCLUSION

1. Soxhlet extraction method had produced higher threshold of gaharu leaf extract than that of maseration method.
2. The highest total phenol concentration was found on extraction method using ethyl acetate solvent.
3. Antibacterial characteristics of gaharu leaf extract was higher toward Gram-positive bacterium than that of Gram-negative bacterium.

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CERTIFICATE OF PROOFREADING

This document certifies that the paper listed below has been edited to ensure that the language is clear and free of errors:

"PHENOL COMPOUND CONTENT AND ANTIBACTERIAL ACTIVITY OF GAHARU LEAF EXTRACT PRODUCTS (*Aquilaria malaccensis*)"

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FELIPE AFONSO VIEIRA

PROFESSIONAL TRANSLATOR

PHENOL COMPOUND CONTENT AND ANTIBACTERIAL ACTIVITY OF GAHARU LEAF EXTRACT PRODUCTS (*Aquilaria malaccensis*)

ABSTRACT

Gaharu leaf extract produces yield extraction, phenol compound and antibacterial activity in diverse quantity. The purpose of this research was to investigate the influence of the extraction method and type of solvent on the extractability of polyphenol component and the antibacterial activity of gaharu leaves. Extraction was done through maceration and ~~soxhlet~~ Soxhlet methods by using solvents of hexane, ethyl acetate and ethanol. The extraction result showed that the highest yield value of 18.4% was found on the treatment of a combination of ethanol solvent and soxhlet method. The total content of phenol and tannin of gaharu leaf extract was in the range of 11.2 to 18.62 mg~~.~~ mL^{-1} and 12.82 to 13.41%, respectively. Antibacterial activity of gaharu leaf extract on Gram-positive test of *Staphylococcus aureus* was higher than that of Gram-negative test of *Escherichia coli* having a value of zone of inhibition in the range of 5.33 to 6.33 mm and 4.00 to 5.00 mm, respectively.

Keywords: ethanol, maceration, phenol, solvent, ~~soxhlet~~Soxhlet.

INTRODUCTION

Parwata *et al.*, (2016) reported that gaharu leaf extract contains phenolic compounds (bioflavonoids), and these compounds can prevent the lipid oxidation process by capturing and neutralizing free radicals. Surjanto *et al.*, (2019) added that gaharu leaf extract has a strong category of antioxidant activity with an IC50 value of 56.985µg. Kamonwannasit *et al.* (2013) stated that gaharu leaf also contains tannin, saponin and *cardiac glycosides* compounds, but has no alkaloid. Tannin is a stringent compound having bitter taste due to its polyphenol cluster that capable ~~to bind and to precipitate~~of binding and of precipitating protein. Tannin is water-~~-~~soluble phenol compound and has a molecular weight in the range of 500 to 300 Da.

Polyphenol compound contains a hydroxyl cluster which is easily soluble within the polar solvent. Different number and position of hydroxyl clusters make this compound has a wide spectrum in term of solubility characteristics within the solvent having different polarity levels. Therefore, extraction by using different

solvents will also produce different polyphenol compounds and antioxidant activity measured (Kratchanova *et al.*, 2010).

The extraction process of a substance is not only affected by type and concentration of the solvent, but also by substance size, extraction period and temperature as well as an extraction method. Extraction methods of Soxhlet and ~~maseration~~ maceration have respective advantages and disadvantages in producing extracted substances. Therefore, the research related to the extraction of the substance ~~by~~ using these two methods is important to be implemented.

The purpose of this research was to investigate the influence of the extraction method and type of solvent on the extractability of polyphenol component and the antibacterial activity of gaharu leaves.

METHODOLOGY

Materials

Leaf of gaharu plant (*Aquileia malaccensis*) obtained from Bangka Tengah District, Bangka Belitung Province, folin ciocalte~~a~~u 50%, alcohol, Galic acid, aquadest, gelatine, indogo carmine, kaoline powder, nutrient broth, plate count agar, and extraction solvents of hexane, ethyl acetate, ethanol, *Escherichia coli* and *Staphylococcus aureus.* Chemical materials and tested bacteria were obtained from the Laboratory of Agricultural Chemistry at Sriwijaya University, Indonesia.

Equipments

Soxhlet extractor, Erlenmeyer flask of 1 L, shaker waterbath, rotaporator, spectrophotometer, autoclave, incubator, petri dish, Eppendorf micro pipete, ose needle, and mixer vortex.

Experimental Design

The experimental design used in this study was Randomized Block Design consisting of two treatment factors of extraction methods (A_1 = Soxhlet and A_2 = maceration) and solvent types (B_1 = hexane~~,~~, B_2 = ethyl acetate and B_3 = ethanol). Data will be processed by using analysis of variance (~~Anova~~ANOVA) and treatment having significant effect will be further analyzed by using Honestly Significant Different (HSD) test at 5% level. The observed parameters were yield

extraction (Lubis and Nova, 2013), total phenol (Pourmorad *et al.*, 2006), percent of tannin (Sudarmadji *et al*., 2007), and antibacterial activity (Miller and Shah, 2000).

Soxhlet Extraction (Marnoto *et al*., 2012)

Sample of gaharu leaf powder ~~were~~ was weighed with a magnitude of 40 g with the water content of 6% and size of 40-60 mesh, wrapped with sieving paper and then put into Soxhlet tube. Soxhlet flask ~~were~~ was filled with a solvent having a volume of 250 mL. Soxhlet unit ~~were~~ was set up and equipped with reverse cooler, heated at boiling point temperature of solvent (hexana, 69ºC; ethyl acetate, 77.1ºC; ethanol, 78.4ºC) let the circulation proceeded for 5-6 hours. The produced solution was subsequently evaporated by using *vacuum rotary evaporator* with pressure and temperature according to the solvent until obtained dry extract.

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Maceration Extraction (Yulianty *et al*., 2011)

Sample of gaharu leaf powder ~~were~~ was weighed with a magnitude of 40 g with the water content of 6% and size of 40-60 mesh, put into the vessel (~~maserator~~macerator) and added with solvent according to treatments having a volume of 250mL. ~~Solution~~ The solution ~~were~~ was subsequently ~~maserated~~ macerated for 3 x 24 hours at room temperature and filtrated ~~by~~ using sieve paper. The produced filtrate ~~were~~ was evaporated by using *vacuum rotary evaporator* with pressure and temperature according to the solvent until viscous extract is obtained. This viscous extract was subsequently dried within the oven for 48 hours until dry extract is produced.

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RESULTS AND DISCUSSION

Yield Extraction

Results of yield extraction from two extraction methods showed that the higher the solvent polarity level, the higher the yield magnitude. The highest yield value of gaharu leaf extract was 18.4% (A_1B_3 treatment), and the lowest yield value was 0.73% (A_2B_1 treatment) such as shown in Fig 1A.

Analysis of variance results showed that extraction method, solvent type and their interactions had a significant effect on yield extraction. Results of HSD test was shown in Table 1.

Table 1. HSD test results related to the effect of the extraction method and solvent type on the yield of gaharu leaf extract.

Extraction method	**~~yield~~ Yield (%)**
A2 (Maseration)	3.50a
A1 (Soxhlet)	8.50b
Solvent type	**~~yield~~ Yield (%)**
B1(hexana)	1.41a
B2 (ethyl acetate)	3.40b
B3 (ethanol)	13.20c

Remarks: Numbers followed by the same letter in the same column are not significantly different (at α =5%).

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Results of HSD test (Table 1) showed that the ~~soxhlet~~ Soxhlet extraction method produced higher yield value than that of ~~maseration~~ maceration extraction method. This was due to the fact that the soxhlet method used heating treatment that ~~had effect on~~affected increasing kinetic energy of the solvent. The higher the kinetic energy, the easier the solvent diffusion into cell tissues of gaharu leaf, resulting in a higher quantity of extract. Daud *et al.* (2011) stated that the Soxhlet extraction method had produced higher yield value of guava leaf than that of ~~maseration~~ maceration method with magnitudes of 25.00% and 18.47%, respectively.

Different threshold values of gaharu leaf extract using different solvents were due to differences ~~of~~ in polarity level of respective solvents. The higher the solvent polarity value, the higher the extraction threshold produced, such as shown by HSD test results (Table 1). High solubility is related to the polarity of solvent and polarity of the extracted substance (*like dissolves like*). ~~Polarity~~ The polarity of solvent is indicated by dipole moment, dielectric constant and solubility in water. Ethanol has a higher polarity index than that of ethyl acetate

and hexane so that it had produced a higher threshold than that of ethyl acetate and hexane (Azizahwati *et al*., 2017).

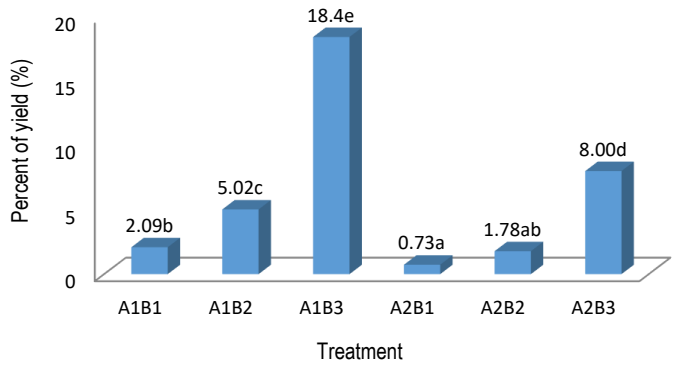

Fig 1A. Extraction yield of gaharu leaf using extraction method of ~~soxhlet~~ Soxhlet (A1) and maceration (A2) with solvents having different polarity levels (B1= hexane, B2 = ethyl acetate and B3 = ethanol).

Results of HSD test showed that treatment interaction of soxhlet extraction method and ethanol solvent had produced the highest yield (Fig 1A). Soxhlet extraction method uses higher temperature that causes lower viscosity of solvent and higher extract solubility, which in turn affect the produced threshold. Ethanol has the highest polarity index than the other solvents; the higher the solvent polarity, the higher the produced extract. Hatam *et al*. (2013) had described that pineapple skin extraction by using soxhlet method and ethanol solvent produced higher total phenol than that of ~~maseration~~ maceration method using the same solvent.

Total Phenol

The highest total phenol with a magnitude of 18.62mg~~.~~ mL^{-1} was found on treatment of Soxhlet extraction method using ethyl acetate solvent~~, whereas~~. In contrast, the lowest one with a magnitude of 9.62 mg~~.~~ mL^{-1} was found on

treatment of ~~maseration~~ maceration extraction method using hexane solvent. Average values of total phenol from gaharu leaf extract was shown in Fig 1B.

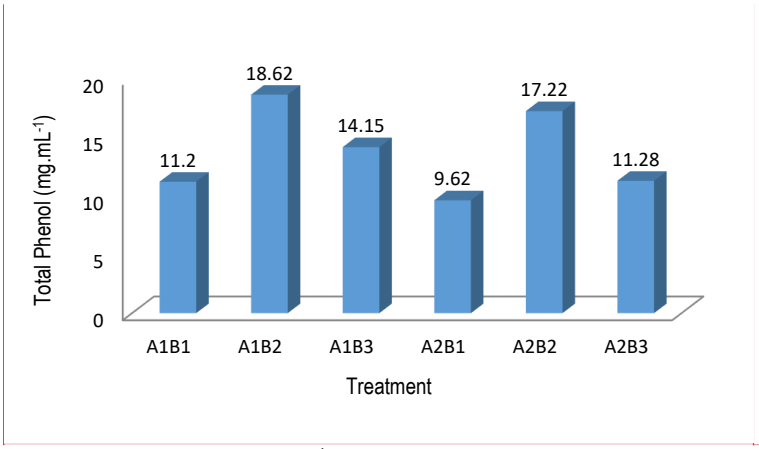

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Fig 1B. Total phenol (mg~~-~~ mL^{-1}) of gaharu leaf produced by using extraction with solvents having different polarity levels.

Results of variance analysis showed that solvent types had a significant effect on the produced total phenol, whereas the interaction of extraction method and solvent types had no significant effect on the produced total phenol. Results of HSD test of solvent types on average values of total phenol was presented in Table 2.

Table 2. Results of HSD test for the effect of solvent types on gaharu leaf yield.

Solvent type	yield (%)
B_1 (hexane)	10.41a
B_3 (ethanol)	12.72a
B_2 (ethyl acetate)	17.92b

Remarks: Numbers followed by the same letters in the same column are not significantly different (at α =5%).

Ethyl acetate solvent produced the highest total phenol than that of other solvents. This is due to the fact that the phenol compound has semipolar characteristic so that it will be more soluble within the semipolar solvent. Semipolar solvents such as ethyl acetate can extract phenol, terpenoid, alkaloid, aglycon and a glycide compounds (Al-Ash'ary *et al.*, 2010). This is in accordance

~~to~~ with the findings of ~~by~~ Hagerman (2002), which showed that phenol compound generally is difficult to precipitate in cold water. Pambayun *et al.* (2007) had added that the highest total phenolate was obtained from gambier extraction process with the Soxhlet method by using ethyl acetate.

Tannin

The highest tannin concentration of gaharu leaf extract with a magnitude of 13.41% was found on treatment of Soxhlet method and ethyl acetate solvent~~, whereas~~. In contrast, the lowest~~-~~ tannin concentration with a magnitude of 12.82% was found on treatment of ~~maseration~~ maceration method and n-hexane solvent. These results were similar to the finding by Ginting *et al.* (2015), which stated that gaharu leaf tea had a bitter taste and contain tannin concentration of 0.2571%. Hadi *et al*. (2011) had added that gaharu leaf extract contains terpenoid, tannin and flavonoid. ~~Average~~ The average value of tannin concentration was shown in Fig 1C.

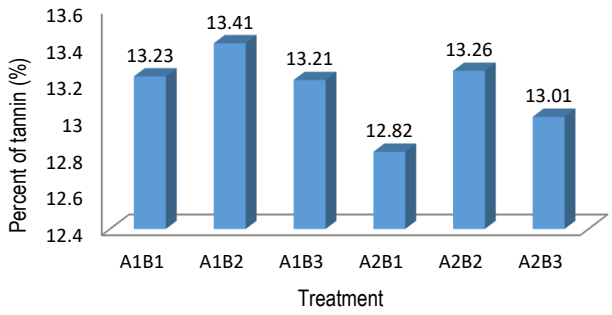

Fig 1C. ~~Percent~~ The percent tannin content of gaharu leaf produced by using extraction methods with solvents having different polarity levels.

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Antibacterial Activity

Antibacterial activity of gaharu leaf extract obtained by using Soxhlet and ~~maseration~~ maceration methods as well as different solvents was explained by the zone of inhibition toward the tested bacteria. ~~Average~~ The average value of the

zone of inhibition toward *Escherichia coli* and *Staphylococcus aureus* bacterium was shown in Fig 2A and Fig 2B.

The use of ethyl acetate solvent in the Soxhlet and maceration extraction methods resulted in ~~more~~ higher zone of inhibition value of both *Escherichia coli* (Fig 2A) and *Staphylococcus aureus* (Fig 2B). This fact was ~~in accordance with~~following the research of Pambayun *et al.*, (2007) stated that the highest antibacterial properties of gambier extract occurred in extracts obtained from extraction using ethyl acetate as a solvent.

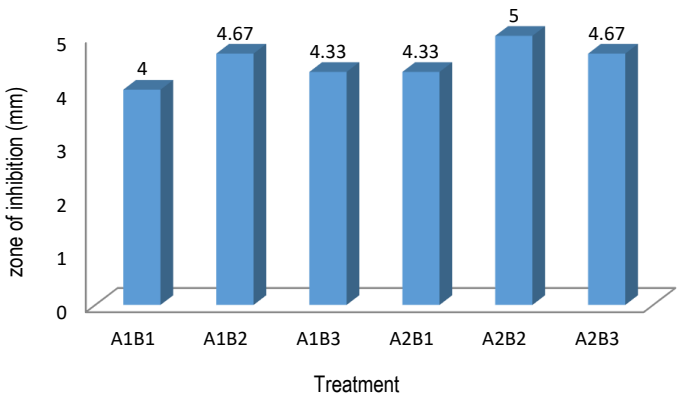

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Fig 2A. The zone of inhibition (mm) value of the bacterial test of *Escherichia coli* on gaharu leaf extract.

Zone of inhibition of gaharu leaf extract toward a bacterial test of *Escherichia coli* (Gram-negative) with a magnitude of 4.67mm was lower than that of the bacterial test of *Staphylococcus aureus* (Gram-positive) with a magnitude of 6.33mm. ~~Capability of gaharu leaf extract as antibacterial agent for Gram-positive and Gram-negative bacteria~~, The capability of gaharu leaf extract as antibacterial agent for Gram-positive and Gram-negative bacteria, is due to phenol and tannin compounds within gaharu leaf. However, the zone of inhibition value of gaharu leaf extract toward Gram-positive bacterium was higher than that of Gram-negative bacterium. This result~~s~~ ~~was~~ were strengthened by

Kamonwannasit *et al.* (2013) finding which stated that gaharu leaf extract was capable ~~to impede~~of impeding *Staphylococcus epidermidis* bacterium with the zone of inhibition value of 12 mm.

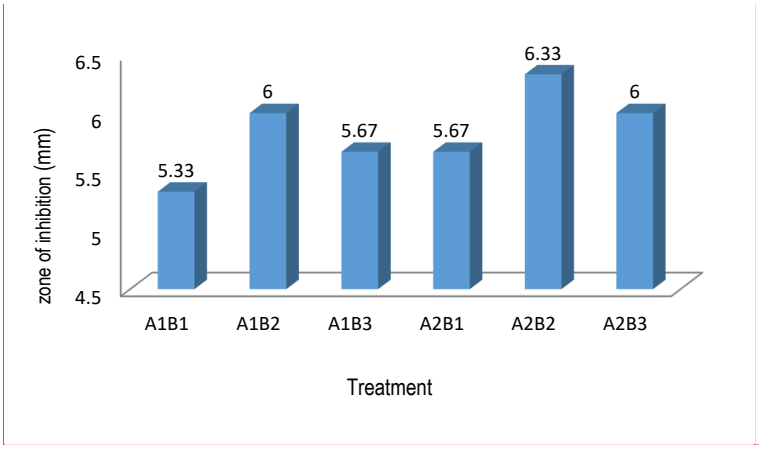

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Fig 2B. The zone of inhibition (mm) value of the bacterial test of *Staphylococcus aureus* on gaharu leaf extract

There are two mechanisms related to bacteria growth obstruction by hydroxide (OH^-) ions, i.e. OH^- ions bind the bacterium cell wall that contains a high concentration of ~~-~~ peptidoglycan, such as found in Gram-positive bacterium. Peptidoglycan found in Gram-positive bacterium is relatively thicker with a magnitude of about 40 nm and located at the surface~~, whereas~~. In contrast, Gram-negative bacterium has thinner peptidoglycan and located ~~at~~ inside. Based on the position of peptidoglycan, peptidoglycan position of Gram-positive bacterium is more accessible by OH^- ions than that of Gram-negative bacterium.

CONCLUSION

1. Soxhlet extraction method had produced a higher threshold of gaharu leaf extract than that of maceration method.
2. The highest total phenol concentration was found on extraction method using ethyl acetate solvent.

3. Antibacterial characteristics of gaharu leaf extract ~~was~~ were higher toward Gram-positive bacterium than that of Gram-negative bacterium.

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Formulir Kiriman Uang

Remittance Application

Penerima/ *Beneficiary* ☐ Penduduk/ *Resident* ☐ Bukan Penduduk/ *Non Resident*

03-Feb-22

Nama/ *Name* : FUNDACAO DE APOIO UNIVERSITARIO

Alamat/ *Address* :

Telepon/ *Phone* :

Kota/ *City* : Negara/ *Country* :

Bank Penerima/ *Beneficiary Bank* : BANCO DO BRAZIL SA

Kota/ *City* : Negara/ *Country* :

No. Rek./ *Acc. No.* : BR0400000000029180000069027C1

Pengirim/ *Remitter* ☐ Penduduk/ *Resident* ☐ Bukan Penduduk/ *Non Resident*

Nama/ *Name* : DR BUDI SANTOSO 109415888

Nama Alias/ *Alias Name* :

No. ID :

KTP/*SIM*/ *Passport*/ *KITAS*

Alamat/ *Address* : PALEMBANG

Telepon/ *Phone* : 081278531631

Kota/ *City* : Negara/ *Country* :

Tujuan Transaksi (*Transaction Purpose*) : .....

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Berita (*Message*) : .....

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Jenis Pengiriman/ *Type of Transfer* : ☐ LLG/ *Clearing* ☐ RTGS ☐ Draft ☐ SWIFT ☐ .....

Sumber Dana/ *Source of fund* :

☐ Tunai/ *Cash* ☐ Cek/ *BG No.* .....

☐ Debit Rek./ *Debit Acc . No.* .....

Mata Uang/ *Currency* : ☐ IDR ☐ USD ☐ .....

Jumlah Dana yang dikirim/ *Amount Transfer* :

Jumlah / *Amount*	Kurs/ *Rate*	Nilai/ *Total Amount*

Biaya/ *Charge*	Valas/ *Amount in Foreign Exchange*	Kurs/ *Amount*	Nilai/ *Total Amount*
Komisi/ *Commission*			
Pengiriman/ *Handling*			
Bank Koresponden / *Correspondent Bank*			
Jumlah Biaya / *Amount Charges* :			
Total yang dibayarkan/ *Total Amount*			

Terbilang/ *Amount in Words* : .....

.....

.....

.....

Biaya dari bank koresponden dibebankan ke rekening/ *Correspondent bank charges are for account of* :

☐ Penerima/ *Beneficiary* ☐ Pengirim/ *Remitter* ☐ Sharing

Saya menyetujui sepenuhnya syarat-syarat yang tercantum pada halaman belakang formulir ini / *I unconditionally accept all the terms and conditions on the reverse form.*

Pejabat Bank/ *Bank Officer* | Teller | Pemohon/ *Applicant*

Sah jika ada cetakan data komputer atau tanda tangan yang berwenang/*The application form will be valid if there is a computerized validation or the authorized signature.*

- Transaksi oleh Walk In Customer (WIC) di atas Rp. 100 juta atau nilai yang setara dengan itu wajib mengisi form PMN (KYC)/ *Transaction by Walk In Customer amounting exceeds Rp. 100.000.000,- (one hundred million rupiahs) or equivalent value must fill in the PMN (KYC) Form .*
- Transaksi oleh bukan penduduk di atas USD 10.000 atau ekuivalen wajib mengisi form LLD1 / *Transactional by non -resident amounting over US $ 10,000 or its equivalent must fill in the LLD 1 Form*

Lembar 3 : Nasabah

EFERENCE : S10PBG00018422

O. TRX. : 62039 947560 96962 TRAN 03/02/2022 10:48:43
O. REK. : 000001000000105 SIMSEM GIRO INTERNAL -
UMLAH : IDR 398,375- 1568
10 - PALEMBANG

O. TRX. : 62039 947560 96962 TRAN 03/02/2022 10:48:43
O. REK. : 010360420801001 PENDAPATAN PROPISI KU
UMLAH : IDR 35,000 1568
10 - PALEMBANG

O. TRX. : 62039 947560 96962 TRAN 03/02/2022 10:48:43
O. REK. : 010360482010001 Pendapatan Restitusi B
UMLAH : IDR 363,375 1568
10 - PALEMBANG

O. TRX. : 62039 947560 96962 TRAN 03/02/2022 10:48:43
O. REK. : 000001000000105 SIMSEM GIRO INTERNAL -
UMLAH : IDR 5,087,250- 1568
10 - PALEMBANG

O. TRX. : 62039 947560 96962 TRAN 03/02/2022 10:48:43
O. REK. : 010840200101001 KU YAKIR
UMLAH : USD 350 1568
10 - PALEMBANG

PT. BANK NEGARA INDONESIA (Persero), Tbk
CABANG : PALEMBANG

IBOC - Maintenance (S10)

Teller ID : 62039
Date : 03/02/2022
Time : 10:52:49

Sender's Reference:
:20:S10PBG00018422
Bank Operation Code:
:23B:CRED
Value Date/Currency/Interbank Settled Amount:
:32A:220203USD350,
Ordering Customer:
:50K:/109415888
DR BUDI SANTOSO
JL PERUMAHAN RAKYAT KOMPLEK TAMAN
SEMBAYU PALEMBANG INDONESIA
Ordering Institution:
:52A:BNINIDJAXXX
Account With Institution:
:57A:BRASBRRJXXX
Beneficiary Customer:
:59:/BR0400000000029180000069027C1
FUNDACAO DE APOIO UNIVERSITARIO
BRAZIL
Remittance Information:
:70:PEMBAYARAN PENERBITAN ARTIKEL
ILMIAH DALAM JURNAL
Details Of Charges:
:71A:OUR
Sender to Receiver Information:
:72:/ACC/AT YR BRAZIL
//FULL AMOUNT/
CNPJ 21238738000161