Bukti Korespondensi publikasi artikel
JURNAL FARMASI SAINS DAN KOMUNITAS
Vol. 19, No. 1, May 2022, pp. 1-7

Penulis :
Nanik Sulistyani*, Lola Angelita, Nurkhasanah

Artikel disubmit pada 17 September 2020 dengan judul :

ANTIBACTERIAL ACTIVITY OF Parsea americana mill. PEELS EXTRACT AND FRACTION CONTAINING PHENOLIC COMPOUND AGAINST Staphylococcus aureus

Berikut rangkuman korespondensi :

Tanggal	Korespondensi		Hal
	Author	Editor Jurnal	
17 September 2020	Artikel disubmit melalui sistem	Email dari Editor tentang ucapan terimakasih atas pengiriman artikel (lampiran 1. Bukti email)	3
2 Nopember 2020	Email tentang informasi sudah upload revisi artikel (Lampiran 2. Bukti email dan lampiran artikel)	Pada portal jurnal ada permintaan revisi setelah initial review	4
2 Desember 2020	Email tentang informasi sudah upload revisi artikel (Lampiran 3. Bukti email dan lampiran artikel)	Pada portal jurnal ada permintaan revisi setelah review berikutnya	16
6 april 2021		Email dari editor tentang permintaan revisi lagi (Lampiran 4. Bukti email dan lampiran hasil review)	28
22 april 2021	Penulis submit ertikel revisi di portal sebelum 22 april	Email dari editor tentang permintaan revisi lagi (Lampiran 5. Bukti email dan lampiran bagian artikel yang masih perlu perbaikan pada hasil review)	41
6 Mei 2021		Email dari editor tentang keputusan diterimanya artikel Lampiran 6. Bukti email	43
15 April		Email dari editor tentang proofreading	44

2022		Lampiran 7. Bukti email	
20 april 2022	email pengiriman hasil proofreading dari penulis Lampiran 8. bukti email		45
18 Mei 2022		Email Permintaan final check dari editor Lampiran 9. Bukti email	46

Lampiran 1. Email dari Editor, ucapan terimakasih atas pengiriman artikel

Gmail nanik sulistyani <naniksulistyani@gmail.com>

[JPSC] Submission Acknowledgement

Enade Perdana Istyastono <libusd@gmail.com> 17 September 2020 09.49
Kepada: Nanik Sulistyani <naniksulistyani@gmail.com>

The following message is being delivered on behalf of Journal of Pharmaceutical Sciences and Community (J Pharm Sci Community).

Nanik Sulistyani:

Thank you for submitting the manuscript, "ANTIBACTERIAL ACTIVITY OF Parsea americana mill. PEELS EXTRACT AND FRACTION CONTAINING PHENOLIC COMPOUND AGAINST Staphylococcus aureus" to Jurnal Farmasi Sains dan Komunitas (Journal of Pharmaceutical Sciences and Community). With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

Manuscript URL:
https://e-journal.usd.ac.id/index.php/JFSK/author/submission/2844
Username: naniksulistyani

If you have any questions, please contact me. Thank you for considering this journal as a venue for your work.

Enade Perdana Istyastono
Jurnal Farmasi Sains dan Komunitas (Journal of Pharmaceutical Sciences and Community)

Journal of Pharmaceutical Sciences and Community (J Pharm Sci Community)
To send an email to JPSC, please make sure to use the email:
editorial.jpsc@usd.ac.id or visit our site in:
http://e-journal.usd.ac.id/index.php/JFSK
(Not just clicking the "reply" button).
Thank you.

Lampiran 2. Bukti email dan lampiran artikel

Gmail nanik sulistyani <naniksulistyani@gmail.com>

Manuscript rev 1

nanik sulistyani <naniksulistyani@gmail.com> 2 November 2020 21.48
Kepada: editorial.jpsc@usd.ac.id

Dear Michael Raharja Gani

Thank you for the information about initial review of our article entitled "ANTIBACTERIAL ACTIVITY OF Parsea americana mill. PEELS
EXTRACT AND FRACTION CONTAINING PHENOLIC COMPOUND AGAINST Staphylococcus
aureus".
We have corrected some sentences and conducted a similarity check again to meet the requirements.
Here I send again the article as the attached file. I also resubmitted via the journal web.
Thank you.

Regards
Nanik Sulistyani

2 lampiran

JSFK LOLA nanik nur_rev 1.docx
429K

JSFK LOLA nanik nur_rev 1.pdf
607K

Artikel :

ANTIBACTERIAL ACTIVITY OF *Parsea americana* mill. PEELS EXTRACT AND FRACTION CONTAINING PHENOLIC COMPOUND AGAINST *Staphylococcus aureus*

Lola Angelita[1,2], Nanik Sulistyani[3*)], Nurkhasanah[3]

[1]Post graduate student Faculty of Pharmacy, Universitas Ahmad Dahlan, Campus 3 Janturan, Warungboto, Umbulharjo, Yogyakarta 55164, Indonesia

[2]College of Pharmacy "STIFAR YAPHAR" Semarang, Plamongan Sari, Pedurungan, Semarang, Central Java 50192, Indonesia

[3]Faculty of Pharmacy, Universitas Ahmad Dahlan, Campus 3 Janturan, Warungboto, Umbulharjo, Yogyakarta 55164, Indonesia

* Corresponding author: Nanik Sulistyani
email: naniksulistyani@gmail.com

Author1: Lolaangelita49@gmail.com
Author3: nurkhas@gmail.com

ABSTRACT

Parsea americana mill. is a natural resource that has been studied as an antibacterial agent. The pulp, peel, and seed of Parsea americana mill. have potential as an antibacterial agent. This research aims to determine the antibacterial activity and phenolic content of Parsea americana mill. peels extract and fraction against Staphylococcus aureus. Parsea americana mill. was macerated with 96% ethanol and then fractionated with solvents of different level of polarity such as n-hexane, ethyl acetate, and methanol solvent, respectively. Antibacterial activity was tested by the Kirby-Bauer method to determine the most active fraction in inhibiting the growth of Staphylococcus aureus. Total phenolics content in the extract and fractions were measured spectrometrically according to the Folin-Ciocalteu method and calculated as gallic acid equivalents (GAE). Antibacterial activity test of the 96% ethanol extract, ethyl acetate fractions, and methanol fractions at a concentration of 10% w/v was showed inhibition zone diameter. At the same time, the n-hexane fraction showed no inhibition zone diameter. The highest inhibition zone is an ethyl acetate fraction as 8.33 ± 0.577 mm. Ethyl acetate fraction of Parsea americana mill. resulted in the amount of 536.26 ± 14.29 mg GAE/g fraction measured by the Folin-Ciocalteu reagent method. The conclusion is that ethyl acetate fraction is the most active in inhibiting the growth of Staphylococcus aureus and has then the highest phenolic content.

Keywords: *Antibacterial; Parsea americana mill. Peels; Staphylococcus aureus; Phenolic content*

INTRODUCTION

Avocado, known as the Latin name *Parsea americana* Mill., is a fruit that has many nutrients and the public widely consumes it. Besides, avocado is widely used as

a mixture of cosmetic ingredients. Utilization of avocado pulp is not proportional to the use of its avocado peels so that the avocado peel are often not used (Fauziah et al., 2016). *Parsea americana* mill. is a natural resources for its antibacterial potential. (Efendi, R, et al., 2019).

Parsea americana mill. is a fruit cultivated in most tropical and subtropical countries. Avocado is a family of Lauraceae with the genus is Parsea, and the species is *Parsea americana* (Doğa Kavaz, 2019). *Parsea americana* Mill. peel, fruit, and leaves are commonly used for the treatment of various diseases such as menorrhagia, hypertension, stomach pain diarrhea, and diabetes (PF et al., 2016). *Parsea americana* Mill. leaf, peel, and seed have biological activities scientifically proven (Amado et al., 2019). In other studies, *Parsea americana* mill. leaf was demonstrated antioxidant activity, antibacterial activity, and total phenolic content of different avocado varieties. The avocado peel extract has been an antibacterial activity against both Gram-positive and Gram-negative bacteria (Amado et al., 2019; Rodríguez-Carpena et al., 2011).

Staphylococcus aureus is a gram-positive bacteria in human skin and mucose but also can cause severe infection (Schmidt et al., 2015). Moreover, *Staphylococcus aureus* is one of the causes of post-operative wound infection, toxic shock syndrome, and food poisoning (Bachir and Benali, 2012). Antibacterial activity of plant extract is the presence of phytochemical compounds such as terpenoids, essential oils, alkaloids, lectins, polypeptides, polyphenolics, and phenol substances (Gonçalves et al.,2005; Cardoso et al., 2016). Phenolic compounds are one of the compounds that have pharmacological activities including antioxidant activities for the prevention and treatment of degenarative disease, cancer, anti-aging, and immune system disorders. Furthermore, Phenolic compounds also retain some therapeutic activity against certain strains of bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Listeria monocytogenes, Pseudomonas sp dan Klebsiella pneumonia* (Rodríguez-Carpena et al., 2011). Phenolic compounds in the *Parsea americana* mill. peel extract is higher than the pulp and seed extract (Amado et al., 2019). Fractionation is a process of separating secondary metabolite compound according to its polarity level. The fractionation process is carried out sequentially, starting from the non-polar solvent to the polar solvent (Purwanto, 2015). Fractionation will show a different total phenolic yield. Determination of phenolic content is a reflection of antibacterial activity (Khoirunnisa et al., 2018). Some of the research conducted were limited to the measurement of total phenolic extract and no studies that conducted total phenolic test

were carried out on *Parsea americana* mill. peel fraction. This research aims to determine the total phenolic content of the extract and fractions of *Parsea americana* mill. peel that will determine the antibacterial potential.

METHODS

Materials and Chemicals

The researched plant sample, which is the peels of *Parsea americana* mill. were collected from Demak, Jawa Tengah, Indonesia. *Staphylococcus aureus* isolates from Institute for Health and Calibration Laboratory Yogyakarta, *Brain Heart Infusion (*Oxoid*)*, *Mueller Hinton Agar (*Oxoid*)* NaCl 0.9% sterile, *Mc Farland* 0.5 (concentration 1.5 x 10^8 CFU/mL), ethanol 96%, n-hexane, ethyl acetate, methanol, paper disk (Oxoid), vancomycin disk (Oxoid), gallic acid (Merck), the Folin-Ciocalteu's phenol reagent (Merck), methanol pro analysis (Merck), sodium carbonate anhydrous (Merck), and bidistilled water.

Extraction and Fractionation

Parsea americana mill. peels were dried in a dryer cabinet at 50ºC for five days then mashed by blending and sieved using mesh 60 to obtain a powder of fruit peel. The dried plant material was extracted by the maceration method. The dry powder of *Parsea americana* mill. peels (200 g) was extracted with 2000 mL ethanol 96% (1:10) then stirred until homogenous then allowed to stand for 24 hours while going to mixing every 4 hours and three times the change of solvent. The macerate was filtered and concentrated using a rotary evaporator (Wulandari et al., 2019). *Parsea americana* mill. extract was suspended in warm water and fractionated using the liquid-liquid extraction method with n-hexane, ethyl acetate, and methanol as solvent, respectively (Putri et al., 2010).

Total Phenolic Content

Total phenolic content of extract and fraction was determined using the Folin-Ciocalteu method as described by (Rohman et al., 2006). The sample was prepared by dissolving 50 mg of the *Parsea americana* mill. extract and fractions using methanol pro analysis in 10 mL and then yield a concentration is obtained 5mg/mL. A total of 200 µL the *Parsea americana* mill.extract and fractions solution is in a test tube filled with 3 mL of bidistiled water. The liquid mixture was added with 0,4 mL of a Folin-Ciocalteau reagent; it was incubated for 5 minutes at 25ºC. The liquid mixture is adding with 4 mL of 7% sodium carbonate, and then add up to 10 mL with bidistiled water. The liquid mixture is shaking gently until homogenous. Absorbance was

measured using a UV-Vis spectrophotometer at 750 nm versus the prepared blank after being incubated for 120 minutes (Rohman et al., 2006).

A calibration curve of standard reference was established using gallic acid (range of concentration from 0.09 to 0.30 mg/mL) as standard references plotted. Total phenolic content of extract and fraction *Parsea americana* mill. was revealed as gallic acid equivalent in milligrams per gram extract or fractions (Rohman et al., 2006).

Media Preparation

Brain Heart Infusion (BHI) media was prepared by dissolving 3.7 g of BHI powder in 100 mL distilled water then stirred until homogeneous. BHI media was sterilized using an autoclave at 121°C for 15 minutes (Broth et al., 2006).

Mueller Hinton Agar (MHA) media was prepared by dissolving 9,5 g of MHA powder in 250 mL distilled water then boil for one minute until complete dissolution and stirred until homogeneous. The media was sterilized using an autoclave at 121°C for 15 minutes (Beef et al., 2015). The MHA solution is warm enough, and then media is poured aseptically into Petri dishes, let stand at room temperature until solidified. Then can be saved at 4°C (in the refrigerator).

Bacterial Preparation

Pure *Staphylococcus aureus* bacteria culture was taken as much as one ose put into 50 mL of BHI media and incubated at 37ºC for 24 hours. A 100 µL of *Staphylococcus aureus* stock was taken and then suspended into 1 mL of BHI media, incubated at 37º C for 1 x 24 hours. Furthermore, the culture of *Staphylococcus aureus* was diluted using 0,9% NaCl sterile until the turbidity was equal to the Mc Farland 0,5 standard (1.5 x 10^8 CFU/mL).

Antibacterial Activity Test

The antibacterial activity test was carried out using the Kirby-Bauer method. A sterile cotton swab was inserted into the bacterial suspension and then evenly rubbed on the MHA media. A total of 20 µL of *Parsea americana* mill. peels extract and fractions with a concentration of 10% were dripped on a 6 mm blank disk paper. The disk paper containing extract and fraction was transferred aseptically to the MHA media that contained the test bacteria and then incubated at 37ºC for 24 hours. The Clear zone formed around the disc indicates the sample can inhibit the growth of bacteria and the diameter can be determined. The lowest concentration of the most active fraction in inhibiting *Staphylococcus aureus* was determined using the same method.

Statistical Analysis

Raw data of total phenolic content and antibacterial effect were tested for normality and homogeneity. The normality test is using the Kolmogorov-Smirnov and Liliefors. Meanwhile, the homogeneity test using the One Way ANOVA and LSD with a confidence level of 95%. If the distribution is normal and homogeneous, then it is continued test by One Way ANOVA with Tukey and LSD and the correlation test is done through the Pearson test. If the data is not normal and not homogenous, then it is continued by the Kruskal-Wallis test and the Mann-Whitney test.

RESULTS AND DISCUSSION

In this research, the extraction of *Parsea americana* mill. peels was prepared with the maceration method. The Maceration method is a very simple extraction method with a long extraction time. The extraction could be used to thermolabile compounds. The ethanol is used as the extraction solvent because it is semi-polar solvent with a polarity index of 5.2 to be able to extract compounds with more distinct polarity (Zuraida et al., 2017). Ethanol is an effective solvent for extracting polyphenol compounds and is safe for human consumption. The ethanol has good extractability because it can penetrate cell walls easily (Dai and Mumper, 2010).

The yield of *Parsea americana* mill. extract with 96% ethanol solvent is 15,81%. The yield value obtained in this research is slightly difference which is 17.04% (Wulandari et al., 2019). The value of a yield shows the effectiveness of an extraction process was influenced by the type of solvent used, the particle size, extraction method, and length of the extraction process (Salamah, et al., 2017). Furthermore, the value of a yield extraction can be influenced by biological factors as plant parts, plant species, harvesting time, and location of growth (Distantina et al., 2009).

Fractionation is a process of separating phytochemical compounds according to polarity level. The fractionation process is carried out sequentially, starting from the non-polar solvent to the polar solvent (Purwanto, 2015). The extract fractionation was carried out using n-hexane, ethyl acetate, and methanol of separate compounds according to their degree of polarity. In this research, the yield of n-hexane, ethyl acetate, and methanol fraction was 27.41%, 8.34%, and 15.83%, respectively. N-hexane can dissolve non-polar compounds such as lipid, wax, aglycon, lignin (Houghton, P.J. and Raman, 1998), sterol, and terpenoid (Cowan, 1999). Ethyl acetate is effective to extract semi-polar compounds such as phenolic, aglycon, and glycoside compounds, and flavonoid (Cowan, 1999; Houghton, P.J. and Raman, 1998).

Methanol can dissolve polar compounds, such as sugar, amino acid, glycoside compounds, phenolic compounds with low and medium molecular weight, and medium polarity (Sri Widyawati et al., 2014).

Antibacterial activity of extract and fractions in *Parsea americana* mill. peels were supported by phytochemical compounds such as total phenol compounds (Sri Widyawati et al., 2014). Total phenolic content of extract and fraction was determined using the Folin-Ciocalteu method. The phenolic compounds of extract and fractions were effective in donating hydrogen atomic to molybdenum ion in Folin-Ciocalteau phenol's reagent so that they resulted in radical phenoxyl stabilizes by resonance or delocalization. The effectivity of phenolic compounds was depended on the type, structure, number, and position of the hydroxyl group of the benzene ring (Wong, et al., 2006, Sri Widyawati et al., 2010;2011;2012;2014). A linear calibration curve of Gallic acid in range 90-300 ug/mL with a coefficient determination (r^2) value of 0.997 was obtained (Figure 1). The total phenolic compounds in the ethyl acetate fraction showed the highest concentration is 536.26 ± 14.29 *mg GAE/g fraction* compared to the other extract and fractions, as shown in (Table 1). Then followed by ethanol extract is 148.72 ± 13.33 *mg GAE/g extract*, methanol fraction is 76.44 ± 4.24 *mg GAE/g fraction,* and n-hexane fraction have the lowest total phenolic compounds is 43.94 ± 3.91 *mg GAE/g fraction.* The total phenolic data shows the extract and fractions in *Parsea americana* mill. peels had polar properties.

The total phenolic compounds of *Parsea americana* mill. pells extract and fraction associated in inhibiting the growth of *Staphylococcus aureus* was determined through antibacterial activity testing using the Kirby-Bauer method. The samples tested include ethanol extract, n-hexane fraction, ethyl acetate fraction, methanol fraction at a concentration of 10% w/v respectively, negative control as 96% ethanol, and positive control as a Vancomycin Antimicrobial Susceptibility Disks 30ug. The result of the antibacterial activity of extract and fraction *Parsea americana* mill. indicated by inhibition zone. The result revealed that ethyl acetate fraction possessed the highest inhibition zone 8.33 ± 0.577 mm of the related fraction presented in (Figure 2). Then followed by ethanol extract is 5.67 ± 0.288 mm, methanol fractions 2.83 ± 0.288 mm and n-hexane fraction does not show inhibitory activity, as shown in (Table 2). In the other research, extract of *Parsea americana* mill. that contains

phytochemical compounds, i.e. flavonoid, saponin, and alkaloid, it can be inhibiting the growth of *Staphylococcus aureus* (Wulandari et al., 2019).

The result of this research, the antibacterial activity of the extract and fractions were observed due to phenolic content. The difference in the total phenolic of each sample is affecting the resulting zone of inhibition. This is evidenced by the higher the total phenolic content, the higher the zone inhibition of *Staphylococcus aureus*. The phenolic compounds will inhibit the growth of Gram-positive bacteria because of the ability to penetrate phenolic compounds on the bacterial cell walls (Purwantiningsih et al., 2014). Phenolic compounds can damage cell membranes, activate the enzyme, and denaturation proteins so that the cell walls are damaged due to decreased permeability. Any change in the permeability of the cytoplasmic membrane will interfere with the transportation of important organic ions into the cell so that it will inhibit growth and even death of cells. High concentrations of phenolic compounds will penetrate and disrupt bacterial cell walls (Oliver et al., 2001).

An antibacterial has activity against bacteria if it has the strength as follows: when providing value inhibition zone with a size of less than 5 mm categorized as weak, 5-10 mm categorized as moderate, 11-20 mm categorized as strong and more than 20 mm means the activity categorized as very strong (Rahmawati et al., 2014). Based on this, the ethyl acetate fractions at a concentration of 10% w/v produces an inhibition zone of 8.33 ± 0.577 mm is including in the medium category.

The result of the statistical analysis shows that antibacterial activity data at extract and fractions of *Parsea americana* mill. is normally distributed and not homogeneous. Based on the normality test of antibacterial activity the significance value >0.05 in the Kolmogorov-Smirnov and Liliefors test with a significance value of 0.200 > 0.05 and then homogeneity test with significance value is 0.012 < 0.05 using One Way ANOVA. So it is necessary to test using the Kruskal-Wallis test and Mann-Whitney test. In the Kruskal-Wallis, test result obtained a significance value 0.005<0.05 and the Mann-Whitney test showed a significant difference from each extract and fraction except there was no significant difference in the n-hexane fraction with ethanol 96% as a negative control. The result of the statistical analysis shows that total phenolic content data is normally distributed and homogeneous with significance value 0.200 > 0.05 in Kolmogorov-Smirnov and Liliefors test and 0.082 > 0.05 using One Way ANOVA, respectively. LSD and Tukey HSD analysis show that the significant value <0.05 there was a significant difference in total phenol

content between the extract and each fraction tested. The correlation test was carried out using the Pearson test and the result showed that the significance value is 0.000 < 0.05. The correlation coefficient value of 0.867 which means there is a correlation between the total phenolic content and antibacterial activity and the value is in the range 0.81-1.00 has a very strong positive correlation where the higher the total phenolic content of extract and fractions are given, the greater the inhibitory zone against *Staphylococcus aureus* bacteria.

CONCLUSION

Total phenolic content of *Parsea americana* mill. peels extract and fractions showed that the highest total phenolic content was 53.63 ± 1.43 *gram of gallic acid equivalent/100g dry material*. Antibacterial activity of *Parsea americana* mill. peels extract and fractions against *Staphylococcus aureus* showed that the ethyl acetate fraction had the inhibition zone was 8.33 ± 0.58 mm compared to other fractions and extract. This research is directly proportional to the higher of the total phenolic concentration, the greater the resulting inhibition zone that appears.

ACKNOWLEDGMENT

Thank you to The Ministry of Education and Culture for the Masters Thesis Research grant for the 2020 budget year.

REFERENCES

Amado, D.A.V., Helmann, G.A.B., Detoni, A.M., de Carvalho, S.L.C., de Aguiar, C.M., Martin, C.A., Tiuman, T.S., Cottica, S.M., 2019. Antioxidant and antibacterial activity and preliminary toxicity analysis of four varieties of avocado (Persea americana Mill.). *Brazilian Journal of Food Technology*, 22, 1–11.

Bachir, R.G., Benali, M., 2012. Antibacterial activity of the essential oils from the leaves of Eucalyptus globulus against Escherichia coli and Staphylococcus aureus. *Asian Pacific Journal of Tropical Biomedicine*, 2(9), 739–742.

Beef, I., Casein, S., Final, A., Agar, M.H., Hinton, M., 2015. 70191 Mueller Hinton Agar (M-H Agar) 493(1979), 70191–70193.

Broth, B.H.I., Heart, B., Broth, G., 2006. Brain Heart Infusion Broth. *Heart*, (April), 13088–13088.

Cowan, M.., 1999. Plant products as antimicrobial agents - PubMed. *Plant Products as antimicrobial agents*, 12(4), 564–82.

Dai, J., Mumper, R.J., 2010. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15(10), 7313–7352.

Distantina, S., Danarto, Y., Fahrurrozi, M., 2009. Pengaruh Kondisi Proses Pada Pengolahan Eucheuma Cottonii Terhadap Rendemen Dan Sifat Gel Karagenan. *Ekuilibrium*, 8(1), 35–40.

Doğa Kavaz, C.O., 2019. Simulation of Lidar-Based Robot Detection Task using

ROS and Gazebo (First received XXXX and in final form XXXX). *European Journal of Science and Technology Special Issue*, (17), XX–XX.
Fauziah, N.A., Saleh, C., Erwin, 2016. Ekstraksi dan uji stabilitas zat warna dari kulit buah alpukat (Persea americana Mill) dengan metode Spektroskopi UV-VIS. *Jurnal Atomik*, 1(1), 12–37.
Houghton, P.J. and Raman, A., 1998. Laboratory Handbook for Fractionation of Natural Extracts. Chapman and Hall, London.
Khoirunnisa, R., Susanti, R., Purwanti, N.U., 2018. Penetapan Kadar Total Flavonoid dan Fenol Fraksi Etil Asetat dari Ekstrak Etanol Rimpang Acorus. SP.
Oliver, S.P., Gillespie, B.E., Lewis, M.J., Ivey, S.J., Almeida, R.A., Luther, D.A., Johnson, D.L., Lamar, K.C., Moorehead, H.D., Dowlen, H.H., 2001. Efficacy of a new premilking teat disinfectant containing a phenolic combination for the prevention of mastitis. *Journal of Dairy Science*, 84(6), 1545–1549.
PF, C., Scarpassa, J., Pretto-Giordano, L., Otaguiri, E., Yamada-Ogatta, S., Nakazato, G., Perugini, M., Moreira, I., Vilas-B鬬s, G., 2016. Antibacterial activity of avocado extracts (Persea americana Mill.) against Streptococcus agalactiae. *Phyton*, 85(1), 218–224.
Pharmaceutical, J.O.F., Activity, A., From, S., Flower, F., Of, P., Frondosa, M., Aktivitas, S., Fraksi, A., Bunga, K., L, M.F., 2019. Original Articel Antibacterial Activity Screening From Fraction Flower 2(1), 38–44.
Purwantiningsih, T.I., Suranindyah, Y.Y., (Widodo), W., 2014. AKTIVITAS SENYAWA FENOL DALAM BUAH MENGKUDU (Morinda citrifolia) SEBAGAI ANTIBAKTERI ALAMI UNTUK PENGHAMBATAN BAKTERI PENYEBAB MASTITIS. *Buletin Peternakan*, 38(1), 59.
Purwanto, S., 2015. Uji Aktivitas Antibakteri Fraksi Aktif Ekstrak Daun Senggani (Melastoma malabathricum L) Terhadap Escherichia coli. *Jurnal Keperawatan Sriwijaya*, 2(2355 5459), 2.
Putri, D., Rachman, A., Mulqie, L., Rismawati, E., Sakti, E., 2010. Uji Aktivitas Antibakteri Fraksi Ekstrak Etanol Biji Alpukat (Persea americana Miller) terhadap Bakteri Propionibacterium acnes 388–393.
Rahmawati, N., Sudjarwo, E., Widodo, E., 2014. Uji aktivitas antibakteri ekstrak herbal terhadap bakteri Escherichia coli. *Jurnal Ilmu-ilmu Peternakan*, 24(3), 24–31.
Rodríguez-Carpena, J.G., Morcuende, D., Andrade, M.J., Kylli, P., Estevez, M., 2011. Avocado (Persea americana Mill.) phenolics, in vitro antioxidant and antimicrobial activities, and inhibition of lipid and protein oxidation in porcine patties. *Journal of Agricultural and Food Chemistry*, 59(10), 5625–5635.
Rohman, A., Riyanto, S., Utari, D., 2006. Antioxidant activities, total phenolic and flavonoid contents of ethyl acetate extract of Mengkudu (Morinda citrifolia, L) fruit and its fractions. *Majalah Farmasi Indonesia*, 17(3), 136–142.
Salamah, M.Sc, Apt., N., Rozak, M., Al Abror, M., 2017. Pengaruh metode penyarian terhadap kadar alkaloid total daun jembirit (Tabernaemontana sphaerocarpa. BL) dengan metode spektrofotometri visibel. *Pharmaciana*, 7(1), 113.
Schmidt, A., Bénard, S., Cyr, S., 2015. Hospital Cost of Staphylococcal Infection after Cardiothoracic or Orthopedic Operations in France: A Retrospective Database Analysis. *Surgical Infections*, 16(4), 428–435.
Sri Widyawati, P., Budianta, T.D.W., Kusuma, F.A., Wijaya, E.L., 2014. Difference of solvent polarity to phytochemical content and antioxidant activity of Pluchea indicia less leaves extracts. *International Journal of Pharmacognosy and Phytochemical Research*, 6(4), 850–855.

Wulandari, G., Abdul Rahman, A., Rubiyanti, R., Studi DIII Jurusan Farmasi, P., Kemenkes Tasikmalaya, P., Email, I., 2019. Uji Aktivitas Antibakteri Ekstrak Etanol Kulit Buah Alpukat (Persea americana Mill) terhadap Staphylococcus aureus ATCC 25923 Antibacterial Activity Of Avocados Peel (Persea americana Mill) Extract On Staphylococcus aureus ATCC 25923. *Media Informasi*, 15, 74–80.

Zuraida, Z., Sulistiyani, S., Sajuthi, D., Suparto, I.H., 2017. FENOL, FLAVONOID, DAN AKTIVITAS ANTIOKSIDAN PADA EKSTRAK KULIT BATANG PULAI (Alstonia scholaris R.Br). *Jurnal Penelitian Hasil Hutan*, 35(3), 211–219.

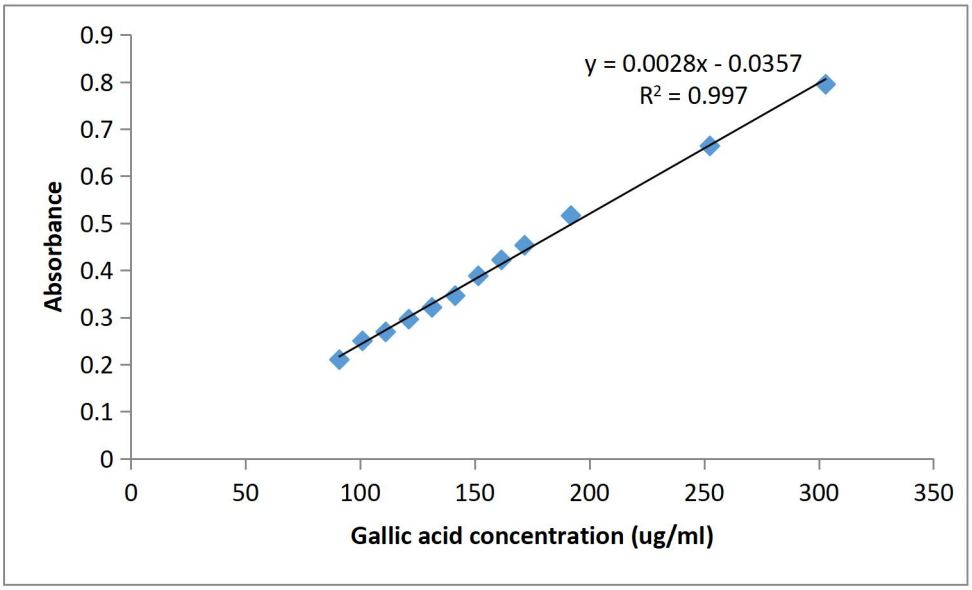


Figure 1. Calibration curve of standard gallic acid for determination of total phenolic content

Table 1. Total phenolic content of extracts and fractions from *Parsea americana* mill.

Sample	Phenolics content (mg GAE/g extract)
Ethanol extract	145.72 ± 13.33
N-hexane fraction	43.94 ± 3.91
Ethyl acetate fraction	536.26 ± 14.29
Methanol fraction	76.44 ± 4.24

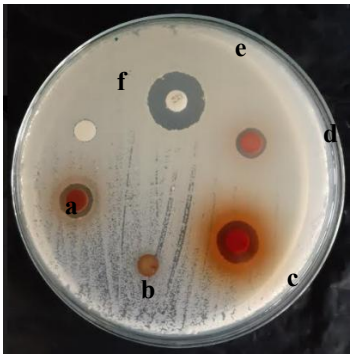


Figure 2. Screening of The Active Faction; (a) 10% ethanol extract, (b) 10% n-hexane fraction, (c) 10% ethyl acetate fraction, (d) 10% methanol fraction, (e) positive control and (f) negative control.

Table 2. Inhibition zone diameter of the extract and fractions against *Staphylococcus aureus.*

Extract and Fractions	Inhibition Zone (mm)	Category
Ethanol Extract (10 % w/v)	5.67±0.288	Weak
n-hexane Fraction (10 % w/v)	0.00±0.00	-
Ethyl Acetate Fraction (10 % w/v)	8.33±0.577	Medium
Methanol Fraction (10 % w/v)	2.83±0.288	Weak
Negative Control (Ethanol 96%)	0,00±0,00	-
Positive Control (Vancomycin 30 µg)	12.17±0.288	Strong

Lampiran 3

Gmail nanik sulistyani <naniksulistyani@gmail.com>

Manuscript rev 1

nanik sulistyani <naniksulistyani@gmail.com> 2 Desember 2020 17.26
Kepada: Michael Raharja Gani <michaelvaynardgani@gmail.com>

[Kutipan teks disembunyikan]

4 lampiran

IMG-20201202-WA0024.jpg
70K

IMG-20201202-WA0025.jpg
186K

JSFK LOLA nanik nur_rev 1.docx
429K

JSFK LOLA nanik nur_rev 1.pdf
607K

Lampiran artikel :

ANTIBACTERIAL ACTIVITY OF *Parsea americana* mill. PEELS EXTRACT AND FRACTION CONTAINING PHENOLIC COMPOUND AGAINST *Staphylococcus aureus*

Lola Angelita[1,2], Nanik Sulistyani[3*)], Nurkhasanah[3]

[1]Post graduate student Faculty of Pharmacy, Universitas Ahmad Dahlan, Campus 3 Janturan, Warungboto, Umbulharjo, Yogyakarta 55164, Indonesia

[2]College of Pharmacy "STIFAR YAPHAR" Semarang, Plamongan Sari, Pedurungan, Semarang, Central Java 50192, Indonesia

[3]Faculty of Pharmacy, Universitas Ahmad Dahlan, Campus 3 Janturan, Warungboto, Umbulharjo, Yogyakarta 55164, Indonesia

* Corresponding author: Nanik Sulistyani
email: naniksulistyani@gmail.com

Author1: Lolaangelita49@gmail.com
Author3: nurkhas@gmail.com

ABSTRACT

Parsea americana mill. is a natural resource that has been studied as an antibacterial agent. The pulp, peel, and seed of Parsea americana mill. have potential as an antibacterial agent. This research aims to determine the antibacterial activity and phenolic content of Parsea americana mill. peels extract and fraction against Staphylococcus aureus. Parsea americana mill. was macerated with 96% ethanol and then fractionated with solvents of different level of polarity such as n-hexane, ethyl acetate, and methanol solvent, respectively. Antibacterial activity was tested by the Kirby-Bauer method to determine the most active fraction in inhibiting the growth of Staphylococcus aureus. Total phenolics content in the extract and fractions were measured spectrometrically according to the Folin-Ciocalteu method and calculated as gallic acid equivalents (GAE). Antibacterial activity test of the 96% ethanol extract, ethyl acetate fractions, and methanol fractions at a concentration of 10% w/v was showed inhibition zone diameter. At the same time, the n-hexane fraction showed no inhibition zone diameter. The highest inhibition zone is an ethyl acetate fraction as 8.33 ± 0.577 mm. Ethyl acetate fraction of Parsea americana mill. resulted in the amount of 536.26 ± 14.29 mg GAE/g fraction measured by the Folin-Ciocalteu reagent method. The conclusion is that ethyl acetate fraction is the most active in inhibiting the growth of Staphylococcus aureus and has then the highest phenolic content.

Keywords: *Antibacterial; Parsea americana mill. Peels; Staphylococcus aureus; Phenolic content*

INTRODUCTION

Avocado, known as the Latin name *Parsea americana* Mill., is a fruit that has many nutrients and the public widely consumes it. Besides, avocado is widely used as

a mixture of cosmetic ingredients. Utilization of avocado pulp is not proportional to the use of its avocado peels so that the avocado peel are often not used (Fauziah et al., 2016). *Parsea americana* mill. is a natural resources for its antibacterial potential. (Efendi, R, et al., 2019).

Parsea americana mill. is a fruit cultivated in most tropical and subtropical countries. Avocado is a family of Lauraceae with the genus is Parsea, and the species is *Parsea americana* (Doğa Kavaz, 2019). *Parsea americana* Mill. peel, fruit, and leaves are commonly used for the treatment of various diseases such as menorrhagia, hypertension, stomach pain diarrhea, and diabetes (PF et al., 2016). *Parsea americana* Mill. leaf, peel, and seed have biological activities scientifically proven (Amado et al., 2019). In other studies, *Parsea americana* mill. leaf was demonstrated antioxidant activity, antibacterial activity, and total phenolic content of different avocado varieties. The avocado peel extract has been an antibacterial activity against both Gram-positive and Gram-negative bacteria (Amado et al., 2019; Rodríguez-Carpena et al., 2011).

Staphylococcus aureus is a gram-positive bacteria in human skin and mucose but also can cause severe infection (Schmidt et al., 2015). Moreover, *Staphylococcus aureus* is one of the causes of post-operative wound infection, toxic shock syndrome, and food poisoning (Bachir and Benali, 2012). Antibacterial activity of plant extract is the presence of phytochemical compounds such as terpenoids, essential oils, alkaloids, lectins, polypeptides, polyphenolics, and phenol substances (Gonçalves et al.,2005; Cardoso et al., 2016). Phenolic compounds are one of the compounds that have pharmacological activities including antioxidant activities for the prevention and treatment of degenarative disease, cancer, anti-aging, and immune system disorders. Furthermore, Phenolic compounds also retain some therapeutic activity against certain strains of bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Listeria monocytogenes, Pseudomonas sp dan Klebsiella pneumonia* (Rodríguez-Carpena et al., 2011). Phenolic compounds in the *Parsea americana* mill. peel extract is higher than the pulp and seed extract (Amado et al., 2019). Fractionation is a process of separating secondary metabolite compound according to its polarity level. The fractionation process is carried out sequentially, starting from the non-polar solvent to the polar solvent (Purwanto, 2015). Fractionation will show a different total phenolic yield. Determination of phenolic content is a reflection of antibacterial activity (Khoirunnisa et al., 2018). Some of the research conducted were limited to the measurement of total phenolic extract and no studies that conducted total phenolic test

were carried out on *Parsea americana* mill. peel fraction. This research aims to determine the total phenolic content of the extract and fractions of *Parsea americana* mill. peel that will determine the antibacterial potential.

METHODS

Materials and Chemicals

The researched plant sample, which is the peels of *Parsea americana* mill. were collected from Demak, Jawa Tengah, Indonesia. *Staphylococcus aureus* isolates from Institute for Health and Calibration Laboratory Yogyakarta, *Brain Heart Infusion (*Oxoid*)*, *Mueller Hinton Agar (*Oxoid*)* NaCl 0.9% sterile, *Mc Farland* 0.5 (concentration 1.5 x 10^8 CFU/mL), ethanol 96%, n-hexane, ethyl acetate, methanol, paper disk (Oxoid), vancomycin disk (Oxoid), gallic acid (Merck), the Folin-Ciocalteu's phenol reagent (Merck), methanol pro analysis (Merck), sodium carbonate anhydrous (Merck), and bidistilled water.

Extraction and Fractionation

Parsea americana mill. peels were dried in a dryer cabinet at 50ºC for five days then mashed by blending and sieved using mesh 60 to obtain a powder of fruit peel. The dried plant material was extracted by the maceration method. The dry powder of *Parsea americana* mill. peels (200 g) was extracted with 2000 mL ethanol 96% (1:10) then stirred until homogenous then allowed to stand for 24 hours while going to mixing every 4 hours and three times the change of solvent. The macerate was filtered and concentrated using a rotary evaporator (Wulandari et al., 2019). *Parsea americana* mill. extract was suspended in warm water and fractionated using the liquid-liquid extraction method with n-hexane, ethyl acetate, and methanol as solvent, respectively (Putri et al., 2010).

Total Phenolic Content

Total phenolic content of extract and fraction was determined using the Folin-Ciocalteu method as described by (Rohman et al., 2006). The sample was prepared by dissolving 50 mg of the *Parsea americana* mill. extract and fractions using methanol pro analysis in 10 mL and then yield a concentration is obtained 5mg/mL. A total of 200 µL the *Parsea americana* mill.extract and fractions solution is in a test tube filled with 3 mL of bidistiled water. The liquid mixture was added with 0,4 mL of a Folin-Ciocalteau reagent; it was incubated for 5 minutes at 25ºC. The liquid mixture is adding with 4 mL of 7% sodium carbonate, and then add up to 10 mL with bidistiled water. The liquid mixture is shaking gently until homogenous. Absorbance was

measured using a UV-Vis spectrophotometer at 750 nm versus the prepared blank after being incubated for 120 minutes (Rohman et al., 2006).

A calibration curve of standard reference was established using gallic acid (range of concentration from 0.09 to 0.30 mg/mL) as standard references plotted. Total phenolic content of extract and fraction *Parsea americana* mill. was revealed as gallic acid equivalent in milligrams per gram extract or fractions (Rohman et al., 2006).

Media Preparation

Brain Heart Infusion (BHI) media was prepared by dissolving 3.7 g of BHI powder in 100 mL distilled water then stirred until homogeneous. BHI media was sterilized using an autoclave at 121°C for 15 minutes (Broth et al., 2006).

Mueller Hinton Agar (MHA) media was prepared by dissolving 9,5 g of MHA powder in 250 mL distilled water then boil for one minute until complete dissolution and stirred until homogeneous. The media was sterilized using an autoclave at 121°C for 15 minutes (Beef et al., 2015). The MHA solution is warm enough, and then media is poured aseptically into Petri dishes, let stand at room temperature until solidified. Then can be saved at 4°C (in the refrigerator).

Bacterial Preparation

Pure *Staphylococcus aureus* bacteria culture was taken as much as one ose put into 50 mL of BHI media and incubated at 37ºC for 24 hours. A 100 µL of *Staphylococcus aureus* stock was taken and then suspended into 1 mL of BHI media, incubated at 37º C for 1 x 24 hours. Furthermore, the culture of *Staphylococcus aureus* was diluted using 0,9% NaCl sterile until the turbidity was equal to the Mc Farland 0,5 standard (1.5 x 10^8 CFU/mL).

Antibacterial Activity Test

The antibacterial activity test was carried out using the Kirby-Bauer method. A sterile cotton swab was inserted into the bacterial suspension and then evenly rubbed on the MHA media. A total of 20 µL of *Parsea americana* mill. peels extract and fractions with a concentration of 10% were dripped on a 6 mm blank disk paper. The disk paper containing extract and fraction was transferred aseptically to the MHA media that contained the test bacteria and then incubated at 37ºC for 24 hours. The Clear zone formed around the disc indicates the sample can inhibit the growth of bacteria and the diameter can be determined. The lowest concentration of the most active fraction in inhibiting *Staphylococcus aureus* was determined using the same method.

Statistical Analysis

Raw data of total phenolic content and antibacterial effect were tested for normality and homogeneity. The normality test is using the Kolmogorov-Smirnov and Liliefors. Meanwhile, the homogeneity test using the One Way ANOVA and LSD with a confidence level of 95%. If the distribution is normal and homogeneous, then it is continued test by One Way ANOVA with Tukey and LSD and the correlation test is done through the Pearson test. If the data is not normal and not homogenous, then it is continued by the Kruskal-Wallis test and the Mann-Whitney test.

RESULTS AND DISCUSSION

In this research, the extraction of *Parsea americana* mill. peels was prepared with the maceration method. The Maceration method is a very simple extraction method with a long extraction time. The extraction could be used to thermolabile compounds. The ethanol is used as the extraction solvent because it is semi-polar solvent with a polarity index of 5.2 to be able to extract compounds with more distinct polarity (Zuraida et al., 2017). Ethanol is an effective solvent for extracting polyphenol compounds and is safe for human consumption. The ethanol has good extractability because it can penetrate cell walls easily (Dai and Mumper, 2010).

The yield of *Parsea americana* mill. extract with 96% ethanol solvent is 15,81%. The yield value obtained in this research is slightly difference which is 17.04% (Wulandari et al., 2019). The value of a yield shows the effectiveness of an extraction process was influenced by the type of solvent used, the particle size, extraction method, and length of the extraction process (Salamah, et al., 2017). Furthermore, the value of a yield extraction can be influenced by biological factors as plant parts, plant species, harvesting time, and location of growth (Distantina et al., 2009).

Fractionation is a process of separating phytochemical compounds according to polarity level. The fractionation process is carried out sequentially, starting from the non-polar solvent to the polar solvent (Purwanto, 2015). The extract fractionation was carried out using n-hexane, ethyl acetate, and methanol of separate compounds according to their degree of polarity. In this research, the yield of n-hexane, ethyl acetate, and methanol fraction was 27.41%, 8.34%, and 15.83%, respectively. N-hexane can dissolve non-polar compounds such as lipid, wax, aglycon, lignin (Houghton, P.J. and Raman, 1998), sterol, and terpenoid (Cowan, 1999). Ethyl acetate is effective to extract semi-polar compounds such as phenolic, aglycon, and glycoside compounds, and flavonoid (Cowan, 1999; Houghton, P.J. and Raman, 1998).

Methanol can dissolve polar compounds, such as sugar, amino acid, glycoside compounds, phenolic compounds with low and medium molecular weight, and medium polarity (Sri Widyawati et al., 2014).

Antibacterial activity of extract and fractions in *Parsea americana* mill. peels were supported by phytochemical compounds such as total phenol compounds (Sri Widyawati et al., 2014). Total phenolic content of extract and fraction was determined using the Folin-Ciocalteu method. The phenolic compounds of extract and fractions were effective in donating hydrogen atomic to molybdenum ion in Folin-Ciocalteau phenol's reagent so that they resulted in radical phenoxyl stabilizes by resonance or delocalization. The effectivity of phenolic compounds was depended on the type, structure, number, and position of the hydroxyl group of the benzene ring (Wong, et al., 2006, Sri Widyawati et al., 2010;2011;2012;2014). A linear calibration curve of Gallic acid in range 90-300 ug/mL with a coefficient determination (r^2) value of 0.997 was obtained (Figure 1). The total phenolic compounds in the ethyl acetate fraction showed the highest concentration is 536.26 ± 14.29 *mg GAE/g fraction* compared to the other extract and fractions, as shown in (Table 1). Then followed by ethanol extract is 148.72 ± 13.33 *mg GAE/g extract*, methanol fraction is 76.44 ± 4.24 *mg GAE/g fraction,* and n-hexane fraction have the lowest total phenolic compounds is 43.94 ± 3.91 *mg GAE/g fraction.* The total phenolic data shows the extract and fractions in *Parsea americana* mill. peels had polar properties.

The total phenolic compounds of *Parsea americana* mill. pells extract and fraction associated in inhibiting the growth of *Staphylococcus aureus* was determined through antibacterial activity testing using the Kirby-Bauer method. The samples tested include ethanol extract, n-hexane fraction, ethyl acetate fraction, methanol fraction at a concentration of 10% w/v respectively, negative control as 96% ethanol, and positive control as a Vancomycin Antimicrobial Susceptibility Disks 30ug. The result of the antibacterial activity of extract and fraction *Parsea americana* mill. indicated by inhibition zone. The result revealed that ethyl acetate fraction possessed the highest inhibition zone 8.33 ± 0.577 mm of the related fraction presented in (Figure 2). Then followed by ethanol extract is 5.67 ± 0.288 mm, methanol fractions 2.83 ± 0.288 mm and n-hexane fraction does not show inhibitory activity, as shown in (Table 2). In the other research, extract of *Parsea americana* mill. that contains

phytochemical compounds, i.e. flavonoid, saponin, and alkaloid, it can be inhibiting the growth of *Staphylococcus aureus* (Wulandari et al., 2019).

The result of this research, the antibacterial activity of the extract and fractions were observed due to phenolic content. The difference in the total phenolic of each sample is affecting the resulting zone of inhibition. This is evidenced by the higher the total phenolic content, the higher the zone inhibition of *Staphylococcus aureus*. The phenolic compounds will inhibit the growth of Gram-positive bacteria because of the ability to penetrate phenolic compounds on the bacterial cell walls (Purwantiningsih et al., 2014). Phenolic compounds can damage cell membranes, activate the enzyme, and denaturation proteins so that the cell walls are damaged due to decreased permeability. Any change in the permeability of the cytoplasmic membrane will interfere with the transportation of important organic ions into the cell so that it will inhibit growth and even death of cells. High concentrations of phenolic compounds will penetrate and disrupt bacterial cell walls (Oliver et al., 2001).

An antibacterial has activity against bacteria if it has the strength as follows: when providing value inhibition zone with a size of less than 5 mm categorized as weak, 5-10 mm categorized as moderate, 11-20 mm categorized as strong and more than 20 mm means the activity categorized as very strong (Rahmawati et al., 2014). Based on this, the ethyl acetate fractions at a concentration of 10% w/v produces an inhibition zone of 8.33 ± 0.577 mm is including in the medium category.

The result of the statistical analysis shows that antibacterial activity data at extract and fractions of *Parsea americana* mill. is normally distributed and not homogeneous. Based on the normality test of antibacterial activity the significance value >0.05 in the Kolmogorov-Smirnov and Liliefors test with a significance value of 0.200 > 0.05 and then homogeneity test with significance value is 0.012 < 0.05 using One Way ANOVA. So it is necessary to test using the Kruskal-Wallis test and Mann-Whitney test. In the Kruskal-Wallis, test result obtained a significance value 0.005<0.05 and the Mann-Whitney test showed a significant difference from each extract and fraction except there was no significant difference in the n-hexane fraction with ethanol 96% as a negative control. The result of the statistical analysis shows that total phenolic content data is normally distributed and homogeneous with significance value 0.200 > 0.05 in Kolmogorov-Smirnov and Liliefors test and 0.082 > 0.05 using One Way ANOVA, respectively. LSD and Tukey HSD analysis show that the significant value <0.05 there was a significant difference in total phenol

content between the extract and each fraction tested. The correlation test was carried out using the Pearson test and the result showed that the significance value is 0.000 < 0.05. The correlation coefficient value of 0.867 which means there is a correlation between the total phenolic content and antibacterial activity and the value is in the range 0.81-1.00 has a very strong positive correlation where the higher the total phenolic content of extract and fractions are given, the greater the inhibitory zone against *Staphylococcus aureus* bacteria.

CONCLUSION

Total phenolic content of *Parsea americana* mill. peels extract and fractions showed that the highest total phenolic content was 53.63 ± 1.43 *gram of gallic acid equivalent/100g dry material*. Antibacterial activity of *Parsea americana* mill. peels extract and fractions against *Staphylococcus aureus* showed that the ethyl acetate fraction had the inhibition zone was 8.33 ± 0.58 mm compared to other fractions and extract. This research is directly proportional to the higher of the total phenolic concentration, the greater the resulting inhibition zone that appears.

ACKNOWLEDGMENT

Thank you to The Ministry of Education and Culture for the Masters Thesis Research grant for the 2020 budget year.

REFERENCES

Amado, D.A.V., Helmann, G.A.B., Detoni, A.M., de Carvalho, S.L.C., de Aguiar, C.M., Martin, C.A., Tiuman, T.S., Cottica, S.M., 2019. Antioxidant and antibacterial activity and preliminary toxicity analysis of four varieties of avocado (Persea americana Mill.). *Brazilian Journal of Food Technology*, 22, 1–11.

Bachir, R.G., Benali, M., 2012. Antibacterial activity of the essential oils from the leaves of Eucalyptus globulus against Escherichia coli and Staphylococcus aureus. *Asian Pacific Journal of Tropical Biomedicine*, 2(9), 739–742.

Beef, I., Casein, S., Final, A., Agar, M.H., Hinton, M., 2015. 70191 Mueller Hinton Agar (M-H Agar) 493(1979), 70191–70193.

Broth, B.H.I., Heart, B., Broth, G., 2006. Brain Heart Infusion Broth. *Heart*, (April), 13088–13088.

Cowan, M.., 1999. Plant products as antimicrobial agents - PubMed. *Plant Products as antimicrobial agents*, 12(4), 564–82.

Dai, J., Mumper, R.J., 2010. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15(10), 7313–7352.

Distantina, S., Danarto, Y., Fahrurrozi, M., 2009. Pengaruh Kondisi Proses Pada Pengolahan Eucheuma Cottonii Terhadap Rendemen Dan Sifat Gel Karagenan. *Ekuilibrium*, 8(1), 35–40.

Doğa Kavaz, C.O., 2019. Simulation of Lidar-Based Robot Detection Task using

ROS and Gazebo (First received XXXX and in final form XXXX). *European Journal of Science and Technology Special Issue*, (17), XX–XX.
Fauziah, N.A., Saleh, C., Erwin, 2016. Ekstraksi dan uji stabilitas zat warna dari kulit buah alpukat (Persea americana Mill) dengan metode Spektroskopi UV-VIS. *Jurnal Atomik*, 1(1), 12–37.
Houghton, P.J. and Raman, A., 1998. Laboratory Handbook for Fractionation of Natural Extracts. Chapman and Hall, London.
Khoirunnisa, R., Susanti, R., Purwanti, N.U., 2018. Penetapan Kadar Total Flavonoid dan Fenol Fraksi Etil Asetat dari Ekstrak Etanol Rimpang Acorus. SP.
Oliver, S.P., Gillespie, B.E., Lewis, M.J., Ivey, S.J., Almeida, R.A., Luther, D.A., Johnson, D.L., Lamar, K.C., Moorehead, H.D., Dowlen, H.H., 2001. Efficacy of a new premilking teat disinfectant containing a phenolic combination for the prevention of mastitis. *Journal of Dairy Science*, 84(6), 1545–1549.
PF, C., Scarpassa, J., Pretto-Giordano, L., Otaguiri, E., Yamada-Ogatta, S., Nakazato, G., Perugini, M., Moreira, I., Vilas-B鬬s, G., 2016. Antibacterial activity of avocado extracts (Persea americana Mill.) against Streptococcus agalactiae. *Phyton*, 85(1), 218–224.
Pharmaceutical, J.O.F., Activity, A., From, S., Flower, F., Of, P., Frondosa, M., Aktivitas, S., Fraksi, A., Bunga, K., L, M.F., 2019. Original Articel Antibacterial Activity Screening From Fraction Flower 2(1), 38–44.
Purwantiningsih, T.I., Suranindyah, Y.Y., (Widodo), W., 2014. AKTIVITAS SENYAWA FENOL DALAM BUAH MENGKUDU (Morinda citrifolia) SEBAGAI ANTIBAKTERI ALAMI UNTUK PENGHAMBATAN BAKTERI PENYEBAB MASTITIS. *Buletin Peternakan*, 38(1), 59.
Purwanto, S., 2015. Uji Aktivitas Antibakteri Fraksi Aktif Ekstrak Daun Senggani (Melastoma malabathricum L) Terhadap Escherichia coli. *Jurnal Keperawatan Sriwijaya*, 2(2355 5459), 2.
Putri, D., Rachman, A., Mulqie, L., Rismawati, E., Sakti, E., 2010. Uji Aktivitas Antibakteri Fraksi Ekstrak Etanol Biji Alpukat (Persea americana Miller) terhadap Bakteri Propionibacterium acnes 388–393.
Rahmawati, N., Sudjarwo, E., Widodo, E., 2014. Uji aktivitas antibakteri ekstrak herbal terhadap bakteri Escherichia coli. *Jurnal Ilmu-ilmu Peternakan*, 24(3), 24–31.
Rodríguez-Carpena, J.G., Morcuende, D., Andrade, M.J., Kylli, P., Estevez, M., 2011. Avocado (Persea americana Mill.) phenolics, in vitro antioxidant and antimicrobial activities, and inhibition of lipid and protein oxidation in porcine patties. *Journal of Agricultural and Food Chemistry*, 59(10), 5625–5635.
Rohman, A., Riyanto, S., Utari, D., 2006. Antioxidant activities, total phenolic and flavonoid contents of ethyl acetate extract of Mengkudu (Morinda citrifolia, L) fruit and its fractions. *Majalah Farmasi Indonesia*, 17(3), 136–142.
Salamah, M.Sc, Apt., N., Rozak, M., Al Abror, M., 2017. Pengaruh metode penyarian terhadap kadar alkaloid total daun jembirit (Tabernaemontana sphaerocarpa. BL) dengan metode spektrofotometri visibel. *Pharmaciana*, 7(1), 113.
Schmidt, A., Bénard, S., Cyr, S., 2015. Hospital Cost of Staphylococcal Infection after Cardiothoracic or Orthopedic Operations in France: A Retrospective Database Analysis. *Surgical Infections*, 16(4), 428–435.
Sri Widyawati, P., Budianta, T.D.W., Kusuma, F.A., Wijaya, E.L., 2014. Difference of solvent polarity to phytochemical content and antioxidant activity of Pluchea indicia less leaves extracts. *International Journal of Pharmacognosy and Phytochemical Research*, 6(4), 850–855.

Wulandari, G., Abdul Rahman, A., Rubiyanti, R., Studi DIII Jurusan Farmasi, P., Kemenkes Tasikmalaya, P., Email, I., 2019. Uji Aktivitas Antibakteri Ekstrak Etanol Kulit Buah Alpukat (Persea americana Mill) terhadap Staphylococcus aureus ATCC 25923 Antibacterial Activity Of Avocados Peel (Persea americana Mill) Extract On Staphylococcus aureus ATCC 25923. *Media Informasi*, 15, 74–80.

Zuraida, Z., Sulistiyani, S., Sajuthi, D., Suparto, I.H., 2017. FENOL, FLAVONOID, DAN AKTIVITAS ANTIOKSIDAN PADA EKSTRAK KULIT BATANG PULAI (Alstonia scholaris R.Br). *Jurnal Penelitian Hasil Hutan*, 35(3), 211–219.

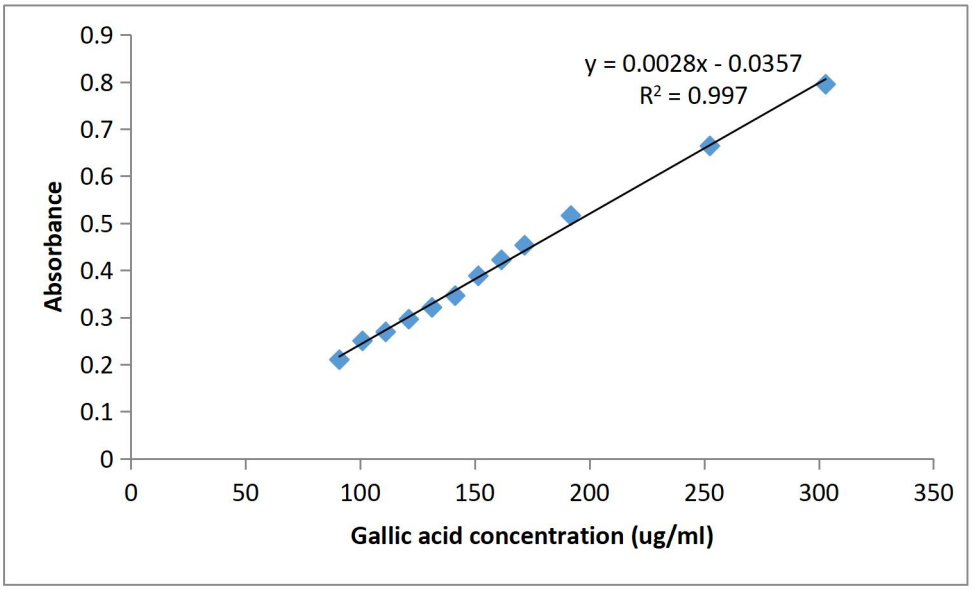


Figure 1. Calibration curve of standard gallic acid for determination of total phenolic content

Table 1. Total phenolic content of extracts and fractions from *Parsea americana* mill.

Sample	Phenolics content (mg GAE/g extract)
Ethanol extract	145.72 ± 13.33
N-hexane fraction	43.94 ± 3.91
Ethyl acetate fraction	536.26 ± 14.29
Methanol fraction	76.44 ± 4.24

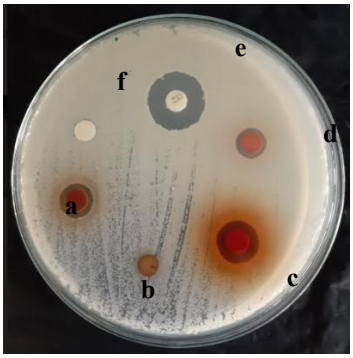


Figure 2. Screening of The Active Faction; (a) 10% ethanol extract, (b) 10% n-hexane fraction, (c) 10% ethyl acetate fraction, (d) 10% methanol fraction, (e) positive control and (f) negative control.

Table 2. Inhibition zone diameter of the extract and fractions against *Staphylococcus aureus.*

Extract and Fractions	Inhibition Zone (mm)	Category
Ethanol Extract (10 % w/v)	5.67±0.288	Weak
n-hexane Fraction (10 % w/v)	0.00±0.00	-
Ethyl Acetate Fraction (10 % w/v)	8.33±0.577	Medium
Methanol Fraction (10 % w/v)	2.83±0.288	Weak
Negative Control (Ethanol 96%)	0,00±0,00	-
Positive Control (Vancomycin 30 µg)	12.17±0.288	Strong

Lampiran 4

Gmail nanik sulistyani <naniksulistyani@gmail.com>

[JPSC] Editor Decision

Michael Raharja Gani <libusd@gmail.com> 6 April 2021 07.14
Kepada: Nanik Sulistyani <naniksulistyani@gmail.com>
Cc: editorial.jpsc@usd.ac.id

Nanik Sulistyani:

We have reached a decision regarding your submission to Jurnal Farmasi Sains dan Komunitas (Journal of Pharmaceutical Sciences and Community), "ANTIBACTERIAL ACTIVITY OF Parsea americana mill. PEELS EXTRACT AND FRACTION CONTAINING PHENOLIC COMPOUND AGAINST Staphylococcus aureus".

Our decision is: Revisions Required

Michael Raharja Gani
Faculty of Pharmacy, Universitas Sanata Dharma
Phone 085643207613
michaelvaynardgani@gmail.com

Reviewer A:

Originality of the paper:
Good

Appropriateness of title/topic:
Good

Appropriateness to J Pharm Sci Community:
Fair

Is the abstract appropriate?:
Fair

Are the keywords appropriate?:
Good

Appropriate research design/methodology
:
Fair

Relevance discussion:
Fair

Valid conclusions:
Fair

Clear, coherent, and well-written manuscript
:
Fair

Quality of English in manuscript:
Fair (required to submit certificate of language proof by professional proofreader)

Appropriate literature citations:
Fair

Quality of figures and tables:
Good

Overall quality of the paper:
Fair

Reviewer B:

Originality of the paper:
Good

Appropriateness of title/topic:
Good

Appropriateness to J Pharm Sci Community:
Good

Is the abstract appropriate?:
Excellent

Are the keywords appropriate?:
Excellent

Appropriate research design/methodology
:
Good

Relevance discussion:
Good

Valid conclusions:
Good

Clear, coherent, and well-written manuscript
:
Good

Quality of English in manuscript:
Good

Appropriate literature citations:
Fair

Quality of figures and tables:
Excellent

Overall quality of the paper:
Good

Comment: there are 3 literatures should be recheck
the wright name: Persea americana Mill.
and please check the notes in document
But overall this is good paper

Journal of Pharmaceutical Sciences and Community (J Pharm Sci Community)
To send an email to JPSC, please make sure to use the email:
editorial.jpsc@usd.ac.id or visit our site in:
http://e-journal.usd.ac.id/index.php/JFSK

(Not just clicking the "reply" button).
Thank you.

3005-Reviewer A.pdf
368K

Hasil review :

JURNAL FARMASI SAINS DAN KOMUNITAS, month year, pp Vol. X No. X
p-ISSN 1693-5683; e-ISSN 2527-7146
doi:

ANTIBACTERIAL ACTIVITY OF *Parsea americana* mill. PEELS EXTRACT AND FRACTION CONTAINING PHENOLIC COMPOUND AGAINST *Staphylococcus aureus*

Commented [II1]: Inhibitory...the test bacteria is only one.

ABSTRACT

Parsea americana mill. is a natural resource that has been studied as an antibacterial agent. The pulp, peel, and seed of Parsea americana mill. have potential as an antibacterial agent. This research aims to determine the ~~antibacterial~~ inhibitory activity and phenolic content of Parsea americana mill. peels extract and fraction against Staphylococcus aureus. Parsea americana mill. was macerated with 96% ethanol and then fractionated with solvents of different level of polarity such as n-hexane, ethyl acetate, and methanol solvent, respectively. Antibacterial activity was tested by the Kirby-Bauer method to determine the most active fraction in inhibiting the growth of Staphylococcus aureus. Total phenolics content in the extract and fractions were measured spectrometrically according to the Folin-Ciocalteu method and calculated as gallic acid equivalents (GAE). Antibacterial activity test of the 96% ethanol extract, ethyl acetate fractions, and methanol fractions at a concentration of 10% w/v was showed inhibition zone ~~diameter~~. At the same time, the n-hexane fraction showed no inhibition zone ~~diameter~~. The highest inhibition zone is an ethyl acetate fraction as 8.33 ± 0.577 mm. The Ethyl acetate fraction of Parsea americana mill. resulted in the amount of 536.26 ± 14.29 mg GAE/g fraction measured by the Folin-Ciocalteu reagent method. The conclusion ~~is~~ was that ethyl acetate fraction is the most active in inhibiting the growth of Staphylococcus aureus and has then the highest phenolic content.

Commented [II2]: ATCC?

Commented [II3]: What is the size of reservoir used?

Keywords: ~~*Antibacterial*~~ Inhibitory activity*; Parsea americana mill. Peels; Staphylococcus aureus; Phenolic content*

INTRODUCTION

Avocado, known as the Latin name *Parsea americana* Mill., is a fruit that has many nutrients and the public widely consumes it. Besides that, avocado is widely used as a mixture of cosmetic ingredients. Utilization of avocado pulp is not proportional to the use of its avocado peels, so that the avocado peel are often not used (Fauziah et al., 2016). *Parsea americana* mill. is a natural resources for its antibacterial potential (Efendi, R, et al., 2019).

Commented [II4]: Pay attention to the writing rules of citation.

Parsea americana mill. is a fruit cultivated in most tropical and subtropical countries. Avocado is a family of Lauraceae with the genus is Parsea, and the species is *Parsea americana* (Doğa Kavaz, 2019). *Parsea americana* Mill. peel, fruit, and leaves are

Commented [II5]: Pay attention to the writing rules of citation.

JURNAL FARMASI SAINS DAN KOMUNITAS, month year, pp Vol. X No. X
p-ISSN 1693-5683; e-ISSN 2527-7146
doi:

ANTIBACTERIAL ACTIVITY OF *Parsea americana* mill. PEELS EXTRACT AND FRACTION CONTAINING PHENOLIC COMPOUND AGAINST *Staphylococcus aureus*

Commented [II1]: Inhibitory...the test bacteria is only one.

ABSTRACT

Parsea americana mill. is a natural resource that has been studied as an antibacterial agent. The pulp, peel, and seed of Parsea americana mill. have potential as an antibacterial agent. This research aims to determine the ~~antibacterial~~ inhibitory activity and phenolic content of Parsea americana mill. peels extract and fraction against Staphylococcus aureus. Parsea americana mill. was macerated with 96% ethanol and then fractionated with solvents of different level of polarity such as n-hexane, ethyl acetate, and methanol solvent, respectively. Antibacterial activity was tested by the Kirby-Bauer method to determine the most active fraction in inhibiting the growth of Staphylococcus aureus. Total phenolics content in the extract and fractions were measured spectrometrically according to the Folin-Ciocalteu method and calculated as gallic acid equivalents (GAE). Antibacterial activity test of the 96% ethanol extract, ethyl acetate fractions, and methanol fractions at a concentration of 10% w/v was showed inhibition zone ~~diameter~~. At the same time, the n-hexane fraction showed no inhibition zone ~~diameter~~. The highest inhibition zone is an ethyl acetate fraction as 8.33 ± 0.577 mm. The *Ethyl acetate fraction of Parsea americana mill. resulted in the amount of 536.26 ± 14.29 mg GAE/g fraction measured by the Folin-Ciocalteu reagent method. The conclusion ~~is~~ was that ethyl acetate fraction is the most active in inhibiting the growth of Staphylococcus aureus and has then the highest phenolic content.*

Commented [II2]: ATCC?

Commented [II3]: What is the size of reservoir used?

Keywords: ~~*Antibacterial*~~ Inhibitory activity*; Parsea americana mill. Peels; Staphylococcus aureus; Phenolic content*

INTRODUCTION

Avocado, known as the Latin name *Parsea americana* Mill., is a fruit that has many nutrients and the public widely consumes it. Besides that, avocado is widely used as a mixture of cosmetic ingredients. Utilization of avocado pulp is not proportional to the use of its avocado peels, so that the avocado peel are often not used (Fauziah et al., 2016). *Parsea americana* mill. is a natural resources for its antibacterial potential (Efendi, R, et al., 2019).

Commented [II4]: Pay attention to the writing rules of citation.

Parsea americana mill. is a fruit cultivated in most tropical and subtropical countries. Avocado is a family of Lauraceae with the genus is Parsea, and the species is *Parsea americana* (Doğa Kavaz, 2019). *Parsea americana* Mill. peel, fruit, and leaves are

Commented [II5]: Pay attention to the writing rules of citation.

The ~~researched plant sample, which is the~~ peels of *Parsea americana* mill. were collected from Demak, Jawa Tengah, Indonesia. *Staphylococcus aureus* isolates obtained from Institute for Health and Calibration Laboratory Yogyakarta, *Brain Heart Infusion (*Oxoid*)*, *Mueller Hinton Agar (*Oxoid*)*, NaCl 0.9% sterile (……?), *Mc Farland* 0.5 (concentration 1.5 x 10^8 CFU/mL) (trademark?), ethanol 96%, n-hexane, ethyl acetate, ~~methanol~~, paper disk (Oxoid), vancomycin disk (Oxoid), gallic acid (Merck), the Folin-Ciocalteu's phenol reagent (Merck), methanol pro analysis (Merck), sodium carbonate anhydrous (Merck), and bidistilled water.

Commented [II10]: From market or cultivation center?

Commented [II11]: ATCC

Commented [II12]: Grade?

Extraction and Fractionation

Parsea americana mill. peels were dried in a dryer cabinet at 50°C for five days then mashed by blending and sieved using mesh 60 to obtain a powder of the fruit peel. ~~The dried plant material was extracted by the maceration method~~. The dry powder of *Parsea americana* mill. peels (200 g) was extracted by maceration method using ~~with~~ 2000 mL ethanol 96% (1:10) then stirred until homogenous then allowed to stand for 24 hours while going to mixing every 4 hours and three times ~~the~~ change of solvent. The macerate was filtered and concentrated using a rotary evaporator (Wulandari et al., 2019). The *Parsea americana* mill. extract was suspended in warm water and fractionated using the liquid-liquid extraction method with n-hexane, ethyl acetate, and methanol as solvent, respectively (Putri et al., 2010).

Total Phenolic Content

The ~~T~~total phenolic content of extract and fractions was determined using the Folin-Ciocalteu method as described by Purwantiningsih et al., (2014). The sample was prepared by dissolving 50 mg of the *Parsea americana* mill. extract and fractions using methanol ~~pro analysis~~ in 10 mL ~~and then yield~~ to obtain concentration ~~is obtained of~~ 5mg/mL. A total of 200 µL ~~the~~ *Parsea americana* mill.extract and fractions solution ~~is~~ was put in a test tube filled with 3 mL of bidistiled water. The liquid mixture was added with 0,4 mL of a Folin-Ciocalteau reagent~~, it was~~ then incubated for 5 minutes at 25°C. The liquid mixture ~~is~~ was ~~adding~~ added with 4 mL of 7% sodium carbonate, and then add up to 10 mL with bidistiled water. The liquid mixture ~~is~~ was shaking gently~~ until homogenous~~. Absorbance was measured using a UV-Vis spectrophotometer at 750 nm ~~versus~~ using the prepared blank after being incubated for 120 minutes (Purwantiningsih et al., 2014).

Commented [II13]: Trademark?

Commented [II14]: Temperature?

The ~~researched plant sample, which is the~~ peels of *Parsea americana* mill. were collected from Demak, Jawa Tengah, Indonesia. *Staphylococcus aureus* isolates obtained from Institute for Health and Calibration Laboratory Yogyakarta, *Brain Heart Infusion (*Oxoid*)*, *Mueller Hinton Agar (*Oxoid*)*, NaCl 0.9% sterile (……?), *Mc Farland* 0.5 (concentration 1.5 x 10^8 CFU/mL) (trademark?), ethanol 96%, n-hexane, ethyl acetate, ~~methanol~~, paper disk (Oxoid), vancomycin disk (Oxoid), gallic acid (Merck), the Folin-Ciocalteu's phenol reagent (Merck), methanol pro analysis (Merck), sodium carbonate anhydrous (Merck), and bidistilled water.

Commented [II10]: From market or cultivation center?

Commented [II11]: ATCC

Commented [II12]: Grade?

Extraction and Fractionation

Parsea americana mill. peels were dried in a dryer cabinet at 50°C for five days then mashed by blending and sieved using mesh 60 to obtain a powder of the fruit peel. ~~The dried plant material was extracted by the maceration method~~. The dry powder of *Parsea americana* mill. peels (200 g) was extracted by maceration method using ~~with~~ 2000 mL ethanol 96% (1:10) then stirred until homogenous then allowed to stand for 24 hours while going to mixing every 4 hours and three times ~~the~~ change of solvent. The macerate was filtered and concentrated using a rotary evaporator (Wulandari et al., 2019). The *Parsea americana* mill. extract was suspended in warm water and fractionated using the liquid-liquid extraction method with n-hexane, ethyl acetate, and methanol as solvent, respectively (Putri et al., 2010).

Total Phenolic Content

The ~~T~~total phenolic content of extract and fractions was determined using the Folin-Ciocalteu method as described by Purwantiningsih et al., (2014). The sample was prepared by dissolving 50 mg of the *Parsea americana* mill. extract and fractions using methanol ~~pro analysis~~ in 10 mL ~~and then yield~~ to obtain concentration ~~is obtained of~~ 5mg/mL. A total of 200 µL ~~the~~ *Parsea americana* mill.extract and fractions solution ~~is~~ was put in a test tube filled with 3 mL of bidistiled water. The liquid mixture was added with 0,4 mL of a Folin-Ciocalteau reagent~~, it was~~ then incubated for 5 minutes at 25°C. The liquid mixture ~~is~~ was ~~adding~~ added with 4 mL of 7% sodium carbonate, and then add up to 10 mL with bidistiled water. The liquid mixture ~~is~~ was shaking gently~~ until homogenous~~. Absorbance was measured using a UV-Vis spectrophotometer at 750 nm ~~versus~~ using the prepared blank after being incubated for 120 minutes (Purwantiningsih et al., 2014).

Commented [II13]: Trademark?

Commented [II14]: Temperature?

Statistical Analysis

Raw data of total phenolic content and antibacterial effect were tested for normality and homogeneity. The normality test ~~is~~ was calculated using the Kolmogorov-Smirnov and Liliefors. Meanwhile, the homogeneity test was determined using the One Way ANOVA and LSD with a confidence level of 95%. If the distribution is normal and homogeneous, then it is continued test by One Way ANOVA with Tukey and LSD and the correlation test is done through the Pearson test. If the data is not normal and not homogenous, then it is continued by the Kruskal-Wallis test and the Mann-Whitney test.

RESULTS AND DISCUSSION

In this research, the extraction of *Parsea americana* mill. peels was prepared with the maceration method. The Maceration method is a very simple extraction method with a long extraction time. The extraction could be used to thermolabile compounds. The ethanol ~~is~~ was used as the extraction solvent because it is semi-polar solvent with a polarity index of 5.2 to be able to extract compounds with more distinct polarity (Zuraida et al., 2017). Ethanol is an effective solvent for extracting polyphenol compounds and is safe for human consumption. The ethanol has good extractability because it can penetrate cell walls easily (Dai and Mumper, 2010).

The yield of *Parsea americana* mill. extract with 96% ethanol solvent ~~is~~ was 15,81%. The yield value obtained in this research is slightly difference which is 17.04% (Wulandari et al., 2019). The ~~value of a~~ yield of the extract ~~shows~~ very closely related to the effectiveness of ~~an~~ extraction process, ~~was~~ which influenced by the type of solvent used, ~~the~~ particle size, extraction method, and length of the extraction process (Salamah, et al., 2017). Furthermore, the ~~value of a~~ yield of extraction can be influenced by biological factors such as plant parts, plant species, harvesting time, and location of growth.

Commented [II21]: Compare to Wulandari?

Fractionation is a process of separating phytochemical compounds according to polarity level. The fractionation process ~~is~~ was carried out sequentially, starting from the non-polar solvent to the polar solvent (Purwanto, 2015). The extract fractionation was carried out using n-hexane, ethyl acetate, and methanol of separate compounds according to their degree of polarity. In this research, the yield of n-hexane, ethyl acetate, and methanol fraction was 27.41%, 8.34%, and 15.83%, respectively. The ~~N~~ n-hexane can dissolve non-polar compounds such as lipid, wax, aglycon, lignin, sterol, and terpenoid. Ethyl acetate is

effective to extract semi-polar compounds such as phenolic, aglycon, and glycoside compounds, and flavonoid. Methanol can dissolve polar compounds, such as sugar, amino acid, glycoside compounds, phenolic compounds with low and medium molecular weight, and medium polarity (Sri Widyawati et al., 2014).

Antibacterial activity of extract and fractions in *Parsea americana* mill. peels were supported by phytochemical compounds such as total phenol compounds (Sri Widyawati et al., 2014). Total phenolic content of extract and fraction was determined using the Folin-Ciocalteu method. The phenolic compounds of extract and fractions were effective in donating hydrogen atomic to molybdenum ion in Folin-Ciocalteau phenol's reagent so that they resulted in radical phenoxyl stabilizes by resonance or delocalization. The effectivity of phenolic compounds was depended on the type, structure, number, and position of the hydroxyl group of the benzene ring (Sri Widyawati et al., 2010;2011;2012;2014). A linear calibration curve of Gallic acid in range 90-300 ug/mL with a coefficient determination (r^2) value of 0.997 was obtained (Figure 1). The total phenolic compounds in the ethyl acetate fraction showed the highest concentration ~~is~~ (536.26 ± 14.29 *mg GAE/g*) *fraction* compared to the other extract and fractions~~, as shown in~~ (Table 1). ~~Then followed by~~ Ethanol, methanol and n-hexane extracts ~~is~~ contained 148.72 ± 13.33 *mg GAE/g extract*~~, methanol fraction is~~ 76.44 ± 4.24 *mg GAE/g fraction,* and 43.94 ± 3.91 *mg GAE/g fraction* ~~n-hexane fraction have the lowest~~ of total phenolic compounds ~~is 43.94 ± 3.91 mg GAE/g fraction~~. Based on the ~~The~~ total phenolic ~~data~~ content, ~~shows~~ the extract and fractions in *Parsea americana* mill. peels had polar properties.

The total phenolic compounds of *Parsea americana* mill. pells extract and fraction growth inhibitory associated ~~in inhibiting the growth of~~ against *Staphylococcus aureus* was determined through antibacterial activity testing using the Kirby-Bauer method. The samples tested include ethanol extract, n-hexane ~~fraction~~, ethyl acetate ~~fraction~~, methanol fraction at a concentration of 10% w/v respectively, negative control ~~as~~ of 96% ethanol, and positive control ~~as a~~ of Vancomycin ~~Antimicrobial Susceptibility Disks~~ 30 ~~ug~~ µg . The result of the antibacterial activity of ~~extract and fraction~~ *Parsea americana* mill. extract and fraction indicated by growth inhibitory ~~inhibition~~ zone. The result revealed that ethyl acetate fraction possessed the highest inhibition zone 8.33 ± 0.577 mm ~~of the related fraction presented in~~ (Figure 2) compared to, while ~~Then followed by~~ the ethanol extract and methanol fractions with diameter ~~is~~ of 5.67 ± 0.288 mm, ~~methanol~~ and 2.83 ± 0.288 mm respectively. ~~And~~ The

Commented [II22]: Pay attention to the writing rules of citation

Commented [II23]: Pay attention to the writing rules of citation

Commented [II24]: Pay attention to the writing rules of citation

Commented [II25]: Configuration figure?

Commented [II26]: How about the diameter of the blank disk?

n-hexane fraction ~~does~~ did not show inhibitory activity~~, as shown in~~ (Table 2). In the ~~other~~ previous research, extract of *Parsea americana* mill. ~~that contains~~ contained phytochemical compounds, i.e. flavonoid, saponin, and alkaloid, ~~it can be inhibiting~~ exhibited ~~the~~ growth inhibitory against ~~of~~ *Staphylococcus aureus* (Wulandari et al., 2019).

The result of this research, the antibacterial activity of the extract and fractions were observed due to phenolic content. The difference in the total phenolic of each sample ~~is affecting~~ affected the resulting zone of inhibition. This ~~is~~ was ~~evidenced~~ proved by the higher the total phenolic content, the higher the zone inhibition diameter of *Staphylococcus aureus*. The phenolic compounds will inhibit the growth of Gram-positive bacteria because of the ability to penetrate phenolic compounds on the bacterial cell walls (Purwantiningsih et al., 2014). Phenolic compounds ~~can~~ damage cell membranes, activate ~~the~~ enzyme, and ~~denaturation~~ denatured proteins so that ~~the~~ damaged cell walls ~~are damaged~~ due to decreased permeability. Any change in the permeability of the cytoplasmic membrane will interfere with the transportation of important organic ions into the cell so that it will inhibit growth and even death of the cells. High concentrations of phenolic compounds will penetrate and disrupt bacterial cell walls (Purwantiningsih et al., 2014).

An antibacterial has activity against bacteria if it has the strength as follows: when providing value inhibition zone with a size of less than 5 mm categorized as weak, 5-10 mm categorized as moderate, 11-20 mm categorized as strong and more than 20 mm means the activity categorized as very strong (Rahmawati et al., 2014). Based on this, the ethyl acetate fractions at a concentration of 10% w/v produces an inhibition zone of 8.33 ± 0.577 mm is including in the medium category. How quantitative data can be accounted for, because the disk is dipped in the test solution? How to ensure that the volume of the extract or fraction sample in each blank disk is the same? Comparison of the inhibitory response should be performed by inserting the same volume of the sample in the disk.

The result of the statistical analysis shows that antibacterial activity data at extract and fractions of *Parsea americana* mill. is normally distributed and not homogeneous. Based on the normality test of antibacterial activity the significance value >0.05 in the Kolmogorov-Smirnov and Liliefors test with a significance value of 0.200 > 0.05 and then homogeneity test with significance value is 0.012 < 0.05 using One Way ANOVA. So it is necessary to test using the Kruskal-Wallis test and Mann-Whitney test. In the Kruskal-Wallis, test result obtained a significance value 0.005<0.05 and the Mann-Whitney test showed a significant

difference from each extract and fraction except there was no significant difference in the n-hexane fraction with ethanol 96% as a negative control. The result of the statistical analysis shows that total phenolic content data is normally distributed and homogeneous with significance value 0.200 > 0.05 in Kolmogorov-Smirnov and Liliefors test and 0.082 > 0.05 using One Way ANOVA, respectively. LSD and Tukey HSD analysis show that the significant value <0.05 there was a significant difference in total phenol content between the extract and each fraction tested. The correlation test was carried out using the Pearson test and the result showed that the significance value is 0.000 < 0.05. The correlation coefficient value of 0.867 which means there is a correlation between the total phenolic content and antibacterial activity and the value is in the range 0.81-1.00 has a very strong positive correlation where the higher the total phenolic content of extract and fractions are given, the greater the inhibitory zone against *Staphylococcus aureus* bacteria.

CONCLUSION

Total phenolic compounds content of *Parsea americana* mill. peels extract and fractions showed that the highest total phenolic content was 53.63 ± 1.43 *gram of gallic acid equivalent/100g dry material*. ~~Antibacterial~~ Inhibitory activity of *Parsea americana* mill. peels extract and fractions against *Staphylococcus aureus* showed that the ethyl acetate fraction had the inhibition zone was 8.33 ± 0.58 mm compared to other fractions and extract. This research is directly proportional to the higher of the total phenolic concentration, the greater the resulting inhibition zone that appears.

Commented [II27]: correct the sentences

ACKNOWLEDGMENT

Thank you to The Ministry of Education and Culture for the Master Thesis Research grant ~~for the~~ 2020 ~~budget year~~.

REFERENCES

Commented [II28]: unformated

Amado, D.A.V., Helmann, G.A.B., Detoni, A.M., de Carvalho, S.L.C., de Aguiar, C.M., Martin, C.A., Tiuman, T.S., Cottica, S.M., 2019. Antioxidant and antibacterial activity and preliminary toxicity analysis of four varieties of avocado (Persea americana Mill.). *Brazilian Journal of Food Technology*, 22, 1–11.

Bachir, R.G., Benali, M., 2012. Antibacterial activity of the essential oils from the leaves of Eucalyptus globulus against Escherichia coli and Staphylococcus aureus. *Asian*

Pacific Journal of Tropical Biomedicine, 2(9), 739–742.

Dai, J., Mumper, R.J., 2010. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15(10), 7313–7352.

Doğa Kavaz, C.O., 2019. Simulation of Lidar-Based Robot Detection Task using ROS and Gazebo (First received XXXX and in final form XXXX). *European Journal of Science and Technology Special Issue*, (17), XX–XX.

Efendi, M.R., 2019. Skrining Aktivitas Antibakteri Fraksi Kelopak Bunga Mussaenda frondosa L. *Journal of Pharmaceutical And Sciences*, 2(1), 38–44.

Fauziah, N.A., Saleh, C., Erwin, 2016. Ekstraksi dan uji stabilitas zat warna dari kulit buah alpukat (Persea americana Mill) dengan metode Spektroskopi UV-VIS. *Jurnal Atomik*, 1(1), 12–37.

Khoirunnisa, R., Susanti, R., Purwanti, N.U., 2018. Penetapan Kadar Total Flavonoid dan Fenol Fraksi Etil Asetat dari Ekstrak Etanol Rimpang Acorus. SP.

PF, C., Scarpassa, J., Pretto-Giordano, L., Otaguiri, E., Yamada-Ogatta, S., Nakazato, G., Perugini, M., Moreira, I., Vilas-B闁s, G., 2016. Antibacterial activity of avocado extracts (Persea americana Mill.) against Streptococcus agalactiae. *Phyton*, 85(1), 218–224.

Purwantiningsih, T.I., Suranindyah, Y.Y., (Widodo), W., 2014. AKTIVITAS SENYAWA FENOL DALAM BUAH MENGKUDU (Morinda citrifolia) SEBAGAI ANTIBAKTERI ALAMI UNTUK PENGHAMBATAN BAKTERI PENYEBAB MASTITIS. *Buletin Peternakan*, 38(1), 59.

Purwanto, S., 2015. Uji Aktivitas Antibakteri Fraksi Aktif Ekstrak Daun Senggani (Melastoma malabathricum L) Terhadap Escherichia coli. *Jurnal Keperawatan Sriwijaya*, 2(2355 5459), 2.

Putri, D., Rachman, A., Mulqie, L., Rismawati, E., Sakti, E., 2010. Uji Aktivitas Antibakteri Fraksi Ekstrak Etanol Biji Alpukat (Persea americana Miller) terhadap Bakteri Propionibacterium acnes 388–393.

Rahmawati, N., Sudjarwo, E., Widodo, E., 2014. Uji aktivitas antibakteri ekstrak herbal terhadap bakteri Escherichia coli. *Jurnal Ilmu-ilmu Peternakan*, 24(3), 24–31.

Rodríguez-Carpena, J.G., Morcuende, D., Andrade, M.J., Kylli, P., Estevez, M., 2011. Avocado (Persea americana Mill.) phenolics, in vitro antioxidant and antimicrobial activities, and inhibition of lipid and protein oxidation in porcine patties. *Journal of Agricultural and Food Chemistry*, 59(10), 5625–5635.

Salamah, M.Sc, Apt., N., Rozak, M., Al Abror, M., 2017. Pengaruh metode penyarian terhadap kadar alkaloid total daun jembirit (Tabernaemontana sphaerocarpa. BL) dengan metode spektrofotometri visibel. *Pharmaciana*, 7(1), 113.

Schmidt, A., Bénard, S., Cyr, S., 2015. Hospital Cost of Staphylococcal Infection after Cardiothoracic or Orthopedic Operations in France: A Retrospective Database Analysis. *Surgical Infections*, 16(4), 428–435.

Sri Widyawati, P., Budianta, T.D.W., Kusuma, F.A., Wijaya, E.L., 2014. Difference of solvent polarity to phytochemical content and antioxidant activity of Pluchea indicia

less leaves extracts. *International Journal of Pharmacognosy and Phytochemical Research*, 6(4), 850–855.

Utomo, S.B., Fujiyanti, M., Lestari, W.P., Mulyani, S., 2018. Antibacterial Activity Test of the C-4-methoxyphenylcalix[4]resorcinarene Compound Modified by Hexadecyltrimethylammonium-Bromide against Staphylococcus aureus and Escherichia coli Bacteria. *JKPK (Jurnal Kimia dan Pendidikan Kimia)*, 3(3), 201.

Wulandari, G., Abdul Rahman, A., Rubiyanti, R., Studi DIII Jurusan Farmasi, P., Kemenkes Tasikmalaya, P., Email, I., 2019. Uji Aktivitas Antibakteri Ekstrak Etanol Kulit Buah Alpukat (Persea americana Mill) terhadap Staphylococcus aureus ATCC 25923 Antibacterial Activity Of Avocados Peel (Persea americana Mill) Extract On Staphylococcus aureus ATCC 25923. *Media Informasi*, 15, 74–80.

Yunus, R., Mongan, R., Rosnani, R., 2017. Cemaran Bakteri Gram Negatif pada Jajanan Siomay di Kota Kendari. *Medical Laboratory Technology Journal*, 3(1), 11.

Zuraida, Z., Sulistiyani, S., Sajuthi, D., Suparto, I.H., 2017. FENOL, FLAVONOID, DAN AKTIVITAS ANTIOKSIDAN PADA EKSTRAK KULIT BATANG PULAI (Alstonia scholaris R.Br). *Jurnal Penelitian Hasil Hutan*, 35(3), 211–219.

Lampiran 5. Email dari editor tentang permintaan revisi lagi

Gmail nanik sulistyani <naniksulistyani@gmail.com>

[JPSC] Editor Decision

Michael Raharja Gani <libusd@gmail.com> 22 April 2021 20.14
Kepada: Nanik Sulistyani <naniksulistyani@gmail.com>
Cc: editorial.jpsc@usd.ac.id

Nanik Sulistyani:

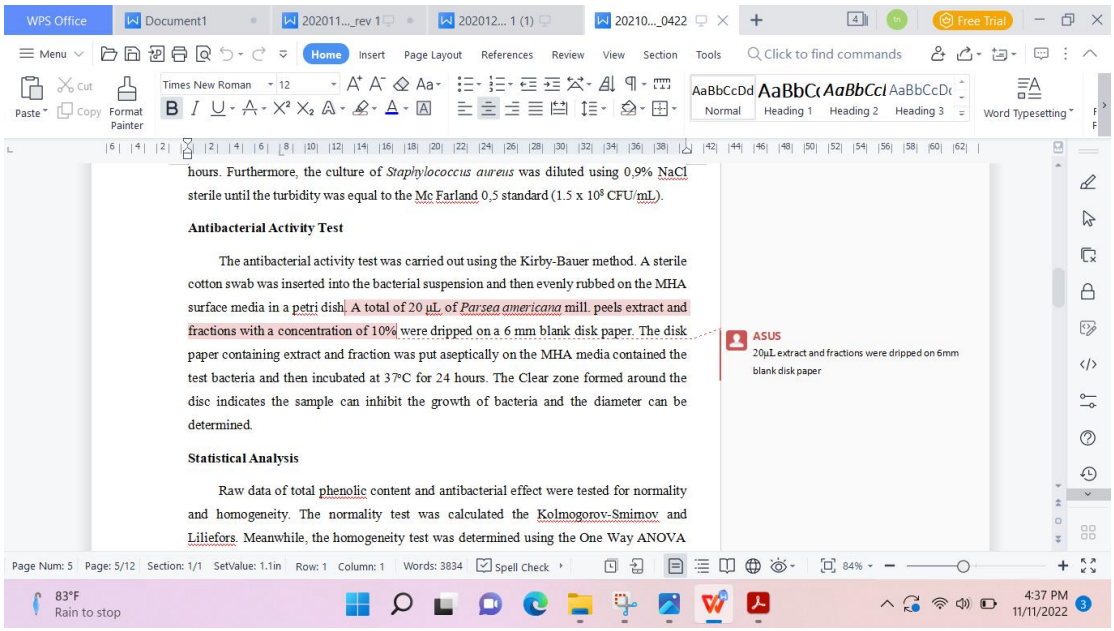
We have reached a decision regarding your submission to Jurnal Farmasi Sains dan Komunitas (Journal of Pharmaceutical Sciences and Community), "ANTIBACTERIAL ACTIVITY OF Parsea americana mill. PEELS EXTRACT AND FRACTION CONTAINING PHENOLIC COMPOUND AGAINST Staphylococcus aureus".

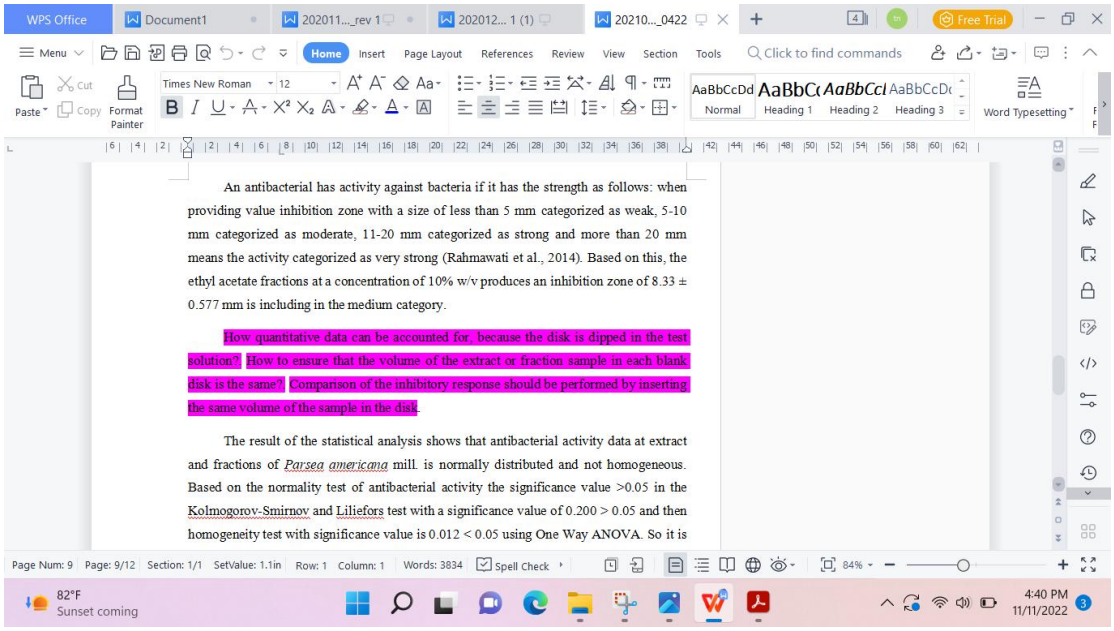
Our decision is: Revisions Required
Here our comment:
1. Introduction - Method doesn't mentioned it.
2. Material and Chemicals - Mc Farland manufacture doesn't mentioned
3. Result and Discussion - Suggestion from reviewer doesn't added
4. Reference - Please ensure all your reference follow our style.

Michael Raharja Gani
Faculty of Pharmacy, Universitas Sanata Dharma
Phone 085643207613
michaelvaynardgani@gmail.com

3005-9207-2-ED_0422.docx
415K

Bagian artikel yang masih perlu perbaikan :





Lampiran 6. Pemberitahuan bahwa artikel diterima

Gmail nanik sulistyani <naniksulistyani@gmail.com>

[JPSC] Editor Decision

Michael Raharja Gani <libusd@gmail.com> 6 Mei 2021 09.39
Kepada: Nanik Sulistyani <naniksulistyani@gmail.com>
Cc: editorial.jpsc@usd.ac.id

Nanik Sulistyani:

We have reached a decision regarding your submission to Jurnal Farmasi Sains dan Komunitas (Journal of Pharmaceutical Sciences and Community), "ANTIBACTERIAL ACTIVITY OF Parsea americana mill. PEELS EXTRACT AND FRACTION CONTAINING PHENOLIC COMPOUND AGAINST Staphylococcus aureus".

Our decision is to: Accept Submission

The copyright assignment form can be accessed here:
http://people.usd.ac.id/~riswanto/jpsc/180413-copyright.doc

Please fill in the form, sign, scan and send it to editorial.jpsc@usd.ac.id before we can continue the further editorial process.

In accordance to the Editorial Policies, you are required to immediately (within two weeks) pay the publication fee of Rp 500,000 (USD 50) by transfer to the following bank account:
Account holder: Lembaga Penelitian Univ. Sanata Dharma
Account No.: 800077540800
Name of the Bank: CIMB Niaga

Please send the proof of remittance by email to the journal administrator (email: dinachristin@usd.ac.id).

Thank you for your valuable contribution to the journal.

Michael Raharja Gani
Faculty of Pharmacy, Universitas Sanata Dharma
Phone 085643207613
michaelvaynardgani@gmail.com

Journal of Pharmaceutical Sciences and Community (J Pharm Sci Community)
To send an email to JPSC, please make sure to use the email:
editorial.jpsc@usd.ac.id or visit our site in:
http://e-journal.usd.ac.id/index.php/JFSK
(Not just clicking the "reply" button).
Thank you.

Lampiran 7. Permintaan proofreading

Gmail nanik sulistyani <naniksulistyani@gmail.com>

[JPSC] Proofreading Request (Author)

Michael Raharja Gani <libusd@gmail.com> 15 April 2022 07.51
Kepada: Nanik Sulistyani <naniksulistyani@gmail.com>
Cc: editorial.jpsc@usd.ac.id

Nanik Sulistyani:

Your submission "ANTIBACTERIAL ACTIVITY OF Parsea americana mill. PEELS EXTRACT AND FRACTION CONTAINING PHENOLIC COMPOUND AGAINST Staphylococcus aureus" to Jurnal Farmasi Sains dan Komunitas (Journal of Pharmaceutical Sciences and Community) now needs to be proofread by folllowing these steps.
1. Click on the Submission URL below.
2. Log into the journal and view PROOFING INSTRUCTIONS
3. Click on VIEW PROOF in Layout and proof the galley in the one or more formats used.
4. Enter corrections (typographical and format) in Proofreading Corrections.
5. Save and email corrections to Layout Editor and Proofreader.
6. Send the COMPLETE email to the editor.

Submission URL:
https://e-journal.usd.ac.id/index.php/JFSK/author/submissionEditing/3005
Username: naniksulistyani

Michael Raharja Gani
Faculty of Pharmacy, Universitas Sanata Dharma
Phone 085643207613
michaelvaynardgani@gmail.com

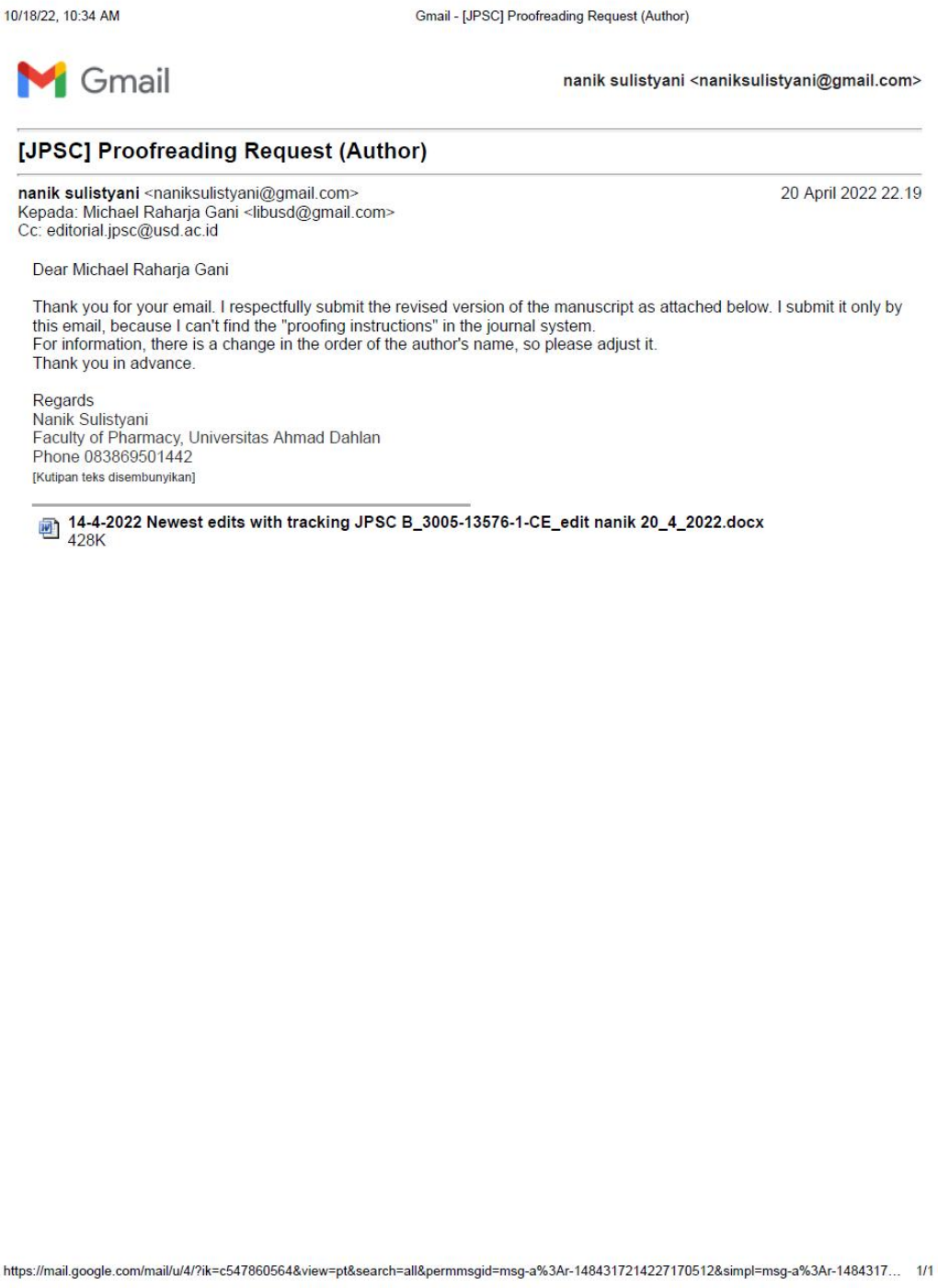
Journal of Pharmaceutical Sciences and Community (J Pharm Sci Community)
To send an email to JPSC, please make sure to use the email:
editorial.jpsc@usd.ac.id or visit our site in:
http://e-journal.usd.ac.id/index.php/JFSK
(Not just clicking the "reply" button).
Thank you.

2 lampiran

14-4-2022 Newest edits with tracking JPSC B_3005-13576-1-CE.docx
432K

14-4-2022 Newest edits without tracking JPSC B_3005-13576-1-CE.docx
409K

Lampiran 8. email pengiriman hasil proofreading dari penulis



10/18/22, 10:34 AM Gmail - [JPSC] Proofreading Request (Author)

Gmail nanik sulistyani <naniksulistyani@gmail.com>

[JPSC] Proofreading Request (Author)

nanik sulistyani <naniksulistyani@gmail.com> 20 April 2022 22.19
Kepada: Michael Raharja Gani <libusd@gmail.com>
Cc: editorial.jpsc@usd.ac.id

Dear Michael Raharja Gani

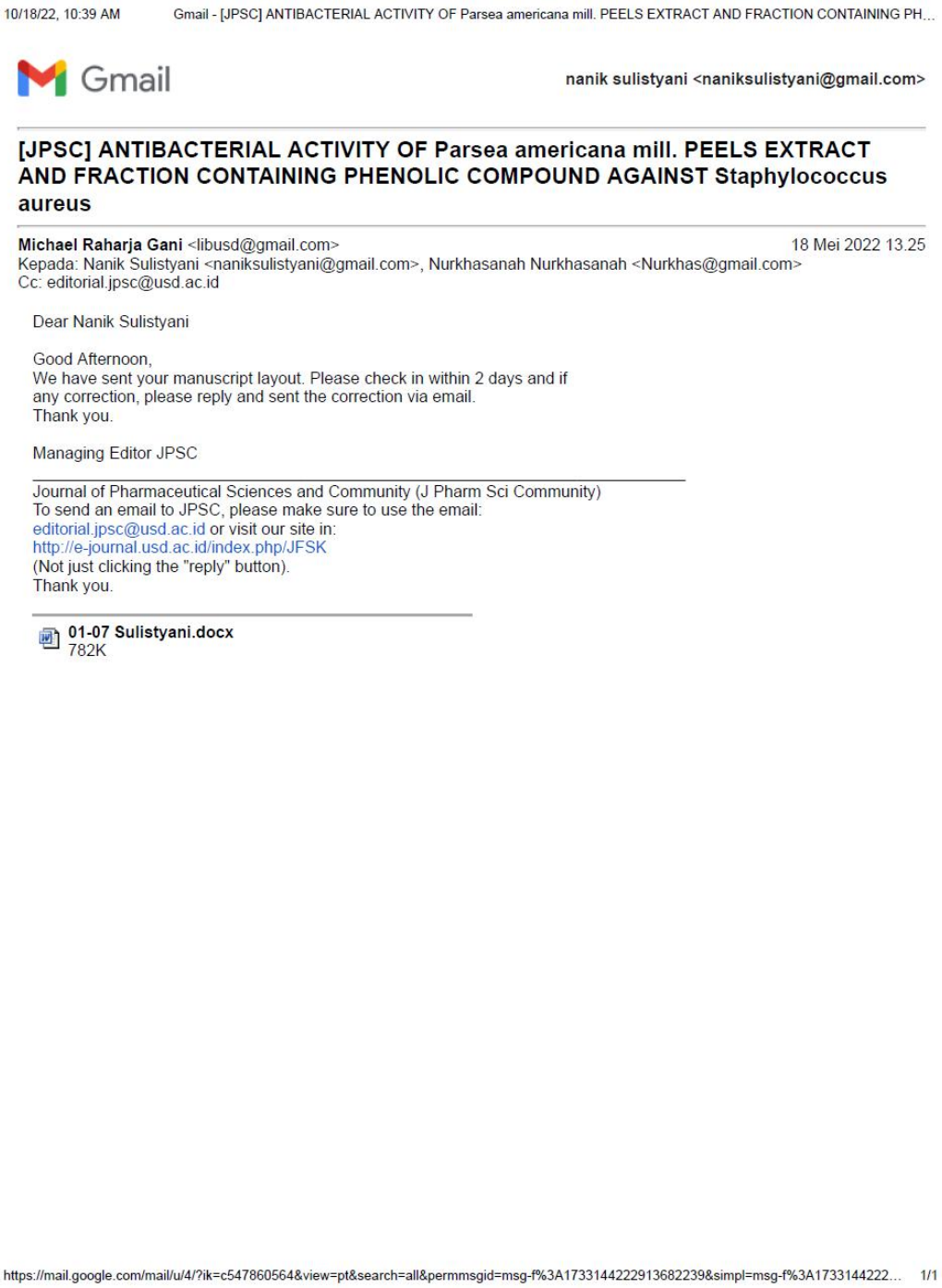
Thank you for your email. I respectfully submit the revised version of the manuscript as attached below. I submit it only by this email, because I can't find the "proofing instructions" in the journal system.
For information, there is a change in the order of the author's name, so please adjust it.
Thank you in advance.

Regards
Nanik Sulistyani
Faculty of Pharmacy, Universitas Ahmad Dahlan
Phone 083869501442
[Kutipan teks disembunyikan]

14-4-2022 Newest edits with tracking JPSC B_3005-13576-1-CE_edit nanik 20_4_2022.docx
428K

https://mail.google.com/mail/u/4/?ik=c547860564&view=pt&search=all&permmsgid=msg-a%3Ar-1484317214227170512&simpl=msg-a%3Ar-1484317… 1/1

Lampiran 9. Email Permintaan final check dari editor



10/18/22, 10:39 AM Gmail - [JPSC] ANTIBACTERIAL ACTIVITY OF Parsea americana mill. PEELS EXTRACT AND FRACTION CONTAINING PH...

Gmail nanik sulistyani <naniksulistyani@gmail.com>

[JPSC] ANTIBACTERIAL ACTIVITY OF Parsea americana mill. PEELS EXTRACT AND FRACTION CONTAINING PHENOLIC COMPOUND AGAINST Staphylococcus aureus

Michael Raharja Gani <libusd@gmail.com> 18 Mei 2022 13.25
Kepada: Nanik Sulistyani <naniksulistyani@gmail.com>, Nurkhasanah Nurkhasanah <Nurkhas@gmail.com>
Cc: editorial.jpsc@usd.ac.id

Dear Nanik Sulistyani

Good Afternoon,
We have sent your manuscript layout. Please check in within 2 days and if any correction, please reply and sent the correction via email.
Thank you.

Managing Editor JPSC

Journal of Pharmaceutical Sciences and Community (J Pharm Sci Community)
To send an email to JPSC, please make sure to use the email:
editorial.jpsc@usd.ac.id or visit our site in:
http://e-journal.usd.ac.id/index.php/JFSK
(Not just clicking the "reply" button).
Thank you.

01-07 Sulistyani.docx
782K

https://mail.google.com/mail/u/4/?ik=c547860564&view=pt&search=all&permmsgid=msg-f%3A1733144222913682239&simpl=msg-f%3A1733144222... 1/1