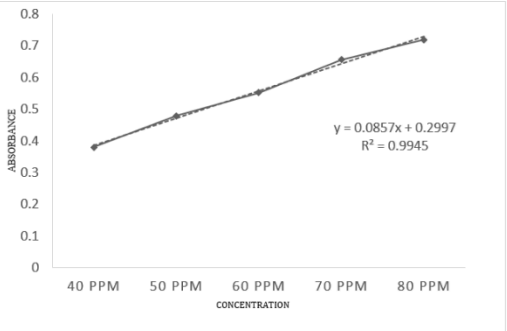
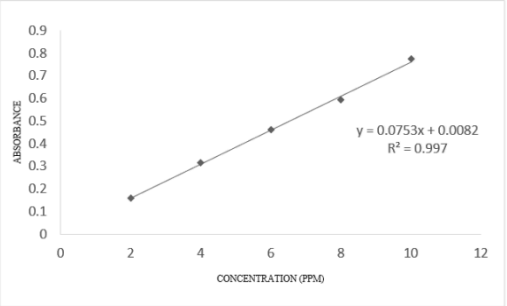
**CORRECTION LIST**

**Manuscript Title**: Standardization, Quantification of Total Phenolics, Flavonoids Content and Antibacterial Activity of Yellow Root (*Arcangelisia flava* (L.) Merr.)

| No | Line/Page/Part | Reviewer Comment or Suggestion | Revision |
|---|---|---|---|
| **Reviewer 1** | | | |
| 1. | Method<br>Page 3 | Please add determination method for plants. | Thank you for the suggestion, we already add determination methods for plants in the material, but now we separated the methods alone.<br>We've revised it by highlighting it in red.<br>**Determination**<br>The plant was identified by Plant Taxonomy Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Indonesia, with No. No.21/HB/03/2021 which stated that the plant used was Arcangelisia flava (L.) Merr. |
| 2. | Method<br>Chromatogram profile<br>Page 4 | Please, each formula should be numbered and cited in paragraphs | Thank you for your suggestions and feedback. We've revised it by highlighting it in red.<br>The developed spot colors were noted, and the Rf values were calculated using formula 1.<br>Retention factor (Rf)=$\frac{distance\ travelled\ by\ the\ solute}{distance\ travelled\ by\ the\ solvent}$ (1) |
| 3. | Method<br>Total flavonoids content (TFC)<br>Page 5 | Showed the wavelength number for the analysis | Thank you for your suggestions and feedback. We've revised it by highlighting it in red.<br>Absorbance was then measured at 435,5 nm |

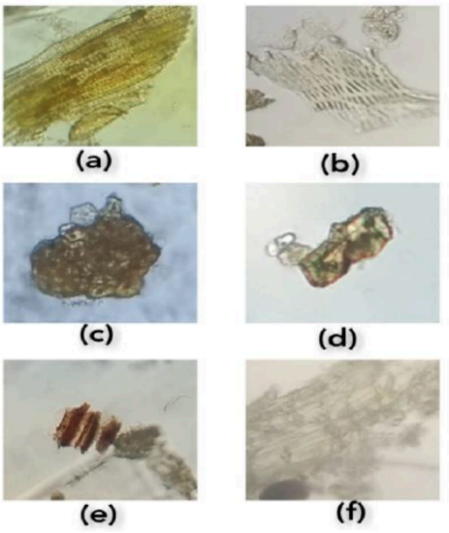
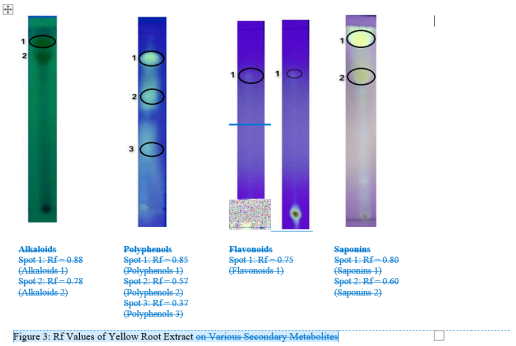
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<td>4.</td>
<td>Result and Disscusion<br>Figure 3<br>Page 9</td>
<td>Please present Rf value in table and move the notation of the picture under the figure title.</td>
<td>Thank you for your suggestions and feedback. We've revised it by highlighting it in red.<br><br>Table 7: Rf Value of Yellow Root Extract on Various Secondary Metabolites<br>
<table>
<tr><th>Metabolites</th><th>Number of Spot</th><th>Rf Value</th></tr>
<tr><td>Alkaloids</td><td>1</td><td>0.88</td></tr>
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<tr><td></td><td>2</td><td>0.57</td></tr>
<tr><td></td><td>3</td><td>0.37</td></tr>
<tr><td>Flavonoids</td><td>1</td><td>0.75</td></tr>
<tr><td>Saponins</td><td>1</td><td>0.80</td></tr>
<tr><td></td><td>2</td><td>0.60</td></tr>
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<td>5</td>
<td>Result and Disscusion<br>Figure 4<br>Page 10</td>
<td>Change in international format, ex: 0,08 to 0.08</td>
<td>Thank you for your suggestions and feedback. We've revised it the figure below.<br>
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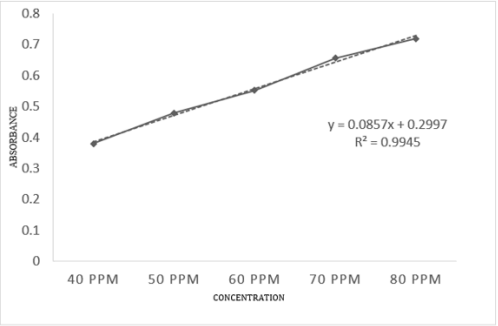
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| 6 | Result and Disscusion<br>Figure 5<br>Page 11 | Change in international format, ex: 0,08 to 0.08 | Thank you for your suggestions and feedback. We've revised it the figure below.<br> |
| **Reviewer 2** | | | |
| 1 | Abstract<br>Page 1 | This section (abstract) lack of conclusion | Thank you for your suggestions and feedback. We've revised it by highlighting it in red.<br>From this research we can obtained that Yellow yellow root extract has a total phenolic content of 87.37 mg GAE/g and flavonoid content of 73.80 mg QE/g. Extract has better antibacterial activity against *Propionibacterium acnes* than its fraction in 800 ppm with diameter inhibition 20.23± 0.58 mm. Standardization of yellow root extract revealed that all parameters matched the criteria requirements. |
| 2 | Abstract<br>Keyword<br>Page 1 | Pls provide the complete taxonomic name of the herb used in this study | Thank you for your suggestions and feedback. We've revised it by highlighting it in red.<br>Keywords: *Arcangelisia flava* (L.) Merr; flavonoids; phenolics; antibacterial; standardization |
| 3 | Introduction<br>Page 1 | Introduction lack of explanation regarding gap, novelty and limitation of previous study. It also lack of justification for selecting P. acnes as subject of antimicrobial test | Thank you for your suggestions and feedback. We've revised it by highlighting it in red.<br>Numerous studies have been conducted on yellow root, particularly pharmacological activity tests, but standardization of yellow root has not been conducted |
| 4 | Introduction<br>Page 2 | This statement doesn't aligned with context of herbal medicine | Thank you for your suggestions and feedback. We've revised it by highlighting it in red.<br>Herbal medicine standardization entails confirming its identity as well as determining its quality and purity. |
| 5 | Method<br>Page 3 | This section lack of analysis data of each method used in this study | Thank you for the suggestion. We have added a detailed methodology in |

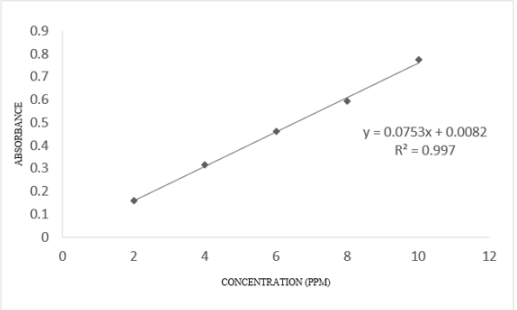
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| | | | accordance with the reviewer's suggestions. |
| 6 | Method<br>Materials<br>Page 3 | Please revise this sentence, as some of the materials were not obtained from the same source. Several purchased from merck, while others from Sigma | Thank you for your suggestions and feedback. We've revised it by highlighting it in red.<br>Methanol, ethanol, hydrochloride acid, acetic acid, sodium hydroxide, sodium ferric chloride, magnesium powder, and aluminium chloride and Silica gel F254 purchased from Merck (Germany). Gallic acid, quercetin, caffein was purchased from Sigma Aldrich (USA) |
| 7 | Method<br>Extraction<br>Page 3 | Please provide a clearer explanation of the term "stable weight" as used in this sentence, as its meaning is biased in the context of this manuscript | Thank you for your suggestions and feedback.<br>Thank you for highlighting this ambiguity. The term “stable weight” was imprecise. The revised text clarifies that rotary evaporation at 40–50 °C was used to obtain a viscous extract, the mass of which was used to calculate the extraction yield. |
| 8 | Method<br>Fractination<br>Page 3 | Please specify whether a liquid or thick (viscous) extract was used. The use of a thick extract is not recommended, due to partial dissolved in aquadest as the solvent. | Thank you for your suggestions and feedback. We've revised it by highlighting it in red.<br>The viscous ethanolic extract (10 g) was mixed with distilled water to obtain a final solution volume of 100 mL. |
| 9 | Method<br>Page 3 | Please add determination method for plants | Thank you for the suggestion, we’ve already add determination methods in this manuscript. We've revised it by highlighting it in red.<br>Determination<br>The plant was identified by Plant Taxonomy Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Indonesia, with No. No.21/HB/03/2021 which stated that the plant used was Arcangelisia flava (L.) Merr. |
| 10 | Method<br>Phytochemical screening<br>Page 4 | Please provide method used for determine presence of these phytochemicals | Thank you for your suggestions and feedback. We've revised it by highlighting it in red.<br>Alkaloid test<br>On a porcelain cup, 100 mg simplicia and viscous extract placed. After that, samples are dissolved in 5 mL HCl 2 M. The obtained solution was divided |

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| | | | into two test tubes. As a blank, three drops of HCl 2 M are added to the first tube. Three drops of Dragendorff reagent are added to the second tube. The Dragendorff reagent will form orange deposits.<br>Phenolic test<br>On test tube, 100 mg simplicia and viscous extract placed. Add 5 mL hot water. Filter up the samples, add with a few drops of 10% iron (III) chloride solution. The presence of tannins is indicated by a dark blue or greenish-black color.<br>Flavonoids test<br>On test tube, 100 mg simplicia and viscous extract placed. Add 5 mL of hot water. Filter up the samples, add 0.05 mg Mg powder and 1 mL concentrated HCl, and vigorously shake. Positive tests produce the colors red, yellow, or orange.<br>Tannin test<br>On test tube, 100 mg simplicia and viscous extract placed. Add 5 mL of hot water. Filter up the samples, add with 1 mL of 1 % gelatine solution. The presence of tannins is indicated by white precipitate.<br>Saponin test<br>On test tube, 100 mg simplicia and viscous extract placed. Add 5 mL of hot water. Filter up the samples, followed by 5 ml of water. The test tube is then vigorously shaken for 10 seconds, and one drop of HCl 2 N is added. A positive test forms a stable froth that reaches a height of 1-10 cm and remains stable for at least 10 minutes.<br>Steroid test<br>On test tube, 100 mg simplicia and viscous extract placed. Add 5 mL of ether. Filter up the samples, added along with two drops of glacial $CH_3COOH$. The solution is gently shaken and set aside for a few minutes. Blue or green indicates steroids.<br>Glycosides test<br>On test tube, 100 mg simplicia and viscous extract placed. Add 5 mL of |

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| | | | ethanol following evaporating. The residue added 1 mL of glacial $CH_3COOH$ and 10 drops of concentrated $H_2SO_4$. Blue or green indicates steroids |
| 11. | Method<br>Chromatogram profile<br>Page 4 | Please, each formula should be numbered and cited in paragraphs | Thank you for your suggestions and feedback. We've revised it by highlighting it in red.<br>The developed spot colors were noted, and the Rf values were calculated using formula 1.<br>Retention factor (Rf)= $\frac{distance\ travelled\ by\ the\ solute}{distance\ travelled\ by\ the\ solvent}$ (1) |
| 12 | Method<br>Total phenolics content (TPC)<br>Page 6 | Pls write down the wavelength of analysis | Thank you for your suggestions and feedback. We've revised it by highlighting it in red.<br><br>After 15 minutes of incubation, the absorbance was recorded at 776.5 nm |
| 13 | Method<br>Total flavonoids content (TFC)<br>Page 7 | Showed the wavelength number for the analysis | Thank you for your suggestions and feedback. We've revised it by highlighting it in red.<br><br>Absorbance was then measured at 435.,5 nmthe maximum absorbance wavelength using a UV-Vis spectrophotometer. |
| 14 | Method<br>Antibacterial Activity Assay<br>Page 7 | Please write all sample used for this assay | Thank you for your suggestions and feedback. We've revised it by highlighting it in red.<br>For each well, 50 µL of solution was added: 25% clindamycin phosphate served as the positive control, 1% DMSO as the negative control, and the experimental groups included plant extract, andor water, ethyl acetate, and n-hexane fractions at 800 ppm and 600 ppm concentrations |
| 15 | Results and discussion<br>Page 8 | Menstrum or menstruum? | Thank you for your suggestions and feedback. We've revised it by highlighting it in red.<br><br>Maceration is an extraction technique where the sample is soaked in a solvent (menstruum) until it is fully immersed. |
| 16 | Results and discussion<br>Table 2: Yield Fraction from | Why the total yield not in 100%? Please give more explanation about this | Thank you for your suggestions and feedback. We've revised it by highlighting it in red.<br>The fraction yield value does not reach 100%, this is likely because the natural |

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| | Yellow Root Extract<br>Page 8 | | raw material consists of various structural components and metabolites. Only certain active compounds are dissolved, while the dregs (such as fiber and complex carbohydrates) are left behind or discarded. |
| 17 | Results and discussion<br>Page 9 | How do you determine the parameters? Also please show the reference related to this | Thank you for your suggestions and feedback. We've revised it by highlighting it in red.<br>The yellow root plant has not been included in the Indonesian herbal pharmacopoeia, so the determination of specific and non-specific parameters is only carried out based on observations by researchers. The results of the form, colour, smell, and taste tests are shown in Table 4. |
| 18 | Results and discussion<br>Page 11<br>Figure 2 | Some of the figures damaged/unclear; therefore cannot be properly used to support the results in this manuscript. | Thank you for your suggestions and feedback. We've revised the picture.<br><br> |
| 19 | Results and discussion<br>Page 13<br>Figure 2<br>Table 7 | Please present Rf value in table and move the notation of the picture under the figure title | Thank you for your suggestions and feedback. We've revised the picture and table by highlighting it in red.<br><br> |

<table>
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<td>20</td>
<td>Results and discussion<br>Page 12<br>Figure 4</td>
<td>Change in international format, ex: 0,08 to 0.08</td>
<td>Thank you for your suggestions and feedback. We've revised the picture as your suggestion.<br><br>
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<tr>
<td>21</td>
<td>Results and discussion<br>Page 13<br>Table 7</td>
<td>Upon calculating the three absorbance values using the provided linear regression equation (y = 0.0857x + 0.2997), the resulting of Total Phenolic Content were inconsistent with those reported in the table.<br>Pls give more explanation of this, as you mentioned in the paragraphs using this linier regresion for determining TPC</td>
<td>Thank you for your suggestions and feedback. We've revised the calculation as your suggestion. I’m really sorry for the mistaken writing, we’ve already changed the mistaken analysis.<br>Table 7: Results of TPC on Yellow Root Extract<br><br>
<table>
<tr><th>Sample</th><th>Replication</th><th>Concentration (ppm)</th><th>Absorbance</th><th>Total Phenolics Content (mg GAE/g)</th></tr>
<tr><td>Extract</td><td>1</td><td>2500</td><td>0.317</td><td>2.523</td></tr>
<tr><td></td><td>2</td><td></td><td>0.321</td><td>3.107</td></tr>
<tr><td></td><td>3</td><td></td><td>0.308</td><td>1.211</td></tr>
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<td>22</td>
<td>Results and discussion<br>Page 12</td>
<td>Change in international format, ex: 0,08 to 0.08</td>
<td>Thank you for your suggestions and feedback. We've revised the picture as your suggestion.</td>
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| | Figure 5 | |  |
| 23 | Results and discussion<br>Page 13<br>Table 9 | Please provide a more in-depth explanation of the results obtained.<br>Some fraction usually exhibit large inhibition zone than crude extract. However the data in this table show the opposite value. | Sorry, I don't understand what is being asked, judging from the table, the inhibition results from the fraction are smaller compared to the extract |
| 25 | Conclusion<br>Page 14 | This conclusion is too short. Pls provide more detailed conclusion related to result in this manuscript | Thank you for your suggestions and feedback. We've revised it by highlighting it in red.<br>From this research we can obtained that yellow root extract has a total phenolic content of 87.37 mg GAE/g and flavonoid content of 73.80 mg QE/g. Extract has better antibacterial activity against Propionibacterium acnes than its fraction in 800 ppm with diameter inhibition 20.23± 0.58 mm<br>Standardization of yellow root extract revealed that all parameters matched the criteria requirements. Extract has high quantities of phenolics and flavonoids. The antibacterial activity from extract was higher from fraction. |
| 26 | Reference<br>Page 15 | Pls used more recent reference (preferably within 10 years) | Thank you for your suggestions and feedback. We've revised it by highlighting it in red.<br>1. Diliarosta S, Sudarmin S S, Efendi A, Dillasamola D, Oktomalioputri B, Ramadhani R. Reconstruction and Scientific Explanation of Akar Kuning (Arcangelisia flava Merr.) From West Sumatra as Ethnomedicine and Source of Science Learning. Pharmacognosy Journal. 2021 Jan 8;13(1):206–11. doi:10.5530/pj.2021.13.29 |

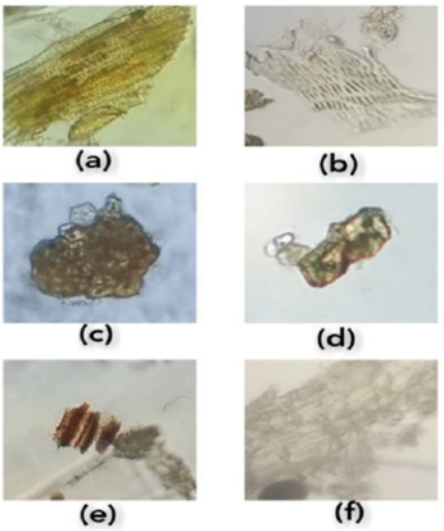
2. Saepudin S, Hidayat TS, Al-Azzahra Y, Cahyati A. Uji Aktivitas Antiinflamasi Ekstrak Batang Akar Kuning (Arcangelisia flava (L.) Merr.) Secara In Vitro. Jurnal Buana Farma. 2024 Mar 26;4(1):1–10. doi:10.36805/jbf.v4i1.934
3. Tavita GE, Lestari D, Linda R, Apindiati RK, Rafdinal R. Phytochemical Testing and In Vitro Anti-inflammatory Activity on Ethanol Extract of Akar Kuning (Arcangelisia flava L) Stems from West Kalimantan. Jurnal Biologi Tropis. 2022;22(4):1334–9.
4. Sakshi Y. Pathrikar, Vaishnavi S.Mukhmale, Nandakishor B. Deshmukh, Swati P. Deshmukh. Standardization extraction and phytochemical screening for herbal drugs. GSC Advanced Research and Reviews. 2025 Jan 30;22(1):084–91. doi:10.30574/gscarr.2025.22.1.0518
5. Yabansabra Y, Bowaire A, Simaremare ES, Yaam DY, Tiris A, Nadeak ESM. Phytochemical and Pharmacological Review of Arcangelisia flava (L.) Merr: Insights into Its Bioactive Compounds and Therapeutic Potential. JURNALBIOLOGIPAPUA. 2025;17(1):82–92.
6. Saptarini NM, Mustarichie R, Herawati IE, Hadisoebroto G. Isolation, Identification, and Quantification of Major Flavonoid In Leaves of Pereskia bleo (Kunth) DC. International Journal of Applied Pharmaceutics. 2022 Nov 26;106–10. doi:10.22159/ijap.2022.v14s4.PP19
7. Herawati IE, Hanifah HN. Antioxidant activity from ethanol extract and fractions of red flame ivy (Hemigraphis colorata Hall. F.) leaf using 1, 1-diphenyl-2-picrylhydrazyl. Drug Invention Today. 2018;10(5):3791–3.
8. Departemen Kesehatan. Parameter Standar Umum Ekstrak Tumbuhan Obat. 2000. 77 p.

9. Kemenkes RI. Farmakope Herbal Indonesia Edisi Kedua. 2nd ed. Jakarta: Departemen Kesehatan Republik Indonesia; 2017. 209–213 p.
10. Nur RM, Dewi R, Kaliu S. Antibacterial activity of methanol extract Rhizophora mucronata leaves toward Salmonella typhi: leading the typhoid fever. Pharmaciana. 2022 Nov 14;12(3):364. doi:10.12928/pharmaciana.v12i3.22475
11. Karthika, S J, S P. TLC and HPTLC Fingerprint Profiles of Different Bioactive Components from the Tuber of Solena amplexicaulis. Journal of Pharmacognosy and Phytochemistry JPP. 2014;3(31):198–206.
12. Hanifah HN, Hadisoebroto G, Dewi L. Comparison of phenolic, flavonoid, and tannin contents from ethanol extract of Kratom stem (Mitragyna speciosa Korth.) and senggani flower (Melastoma malabathrium L.). J Phys Conf Ser. 2021 Apr 1;1869(1):012002. doi:10.1088/1742-6596/1869/1/012002
13. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colometric methods. J Food Drug Anal. 2020 Jul 14;10(3). doi:10.38212/2224-6614.2748
14. Saptarini NM, R. Mustarichie, Aulifa DL, R. Hendriani, Herawati IE. Analysis of Antioxidant and Antibacterial Activity of Leaves Of Fig (Ficus carica L.) From Ciwidey Distric, West Java, Indonesia. RASAYAN Journal of Chemistry. 2022;(Special Issue):172–9. doi:10.31788/RJC.2022.1558205
15. Abubakar AR, Haque M. Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. J Pharm Bioallied Sci. 2020 Jan;12(1):1–10. doi:10.4103/jpbs.JPBS_175_19

16. Bani AA, Amin A, Mun'im A, Radji M. Rasio Nilai Rendamen dan Lama Ekstraksi Maserat Etanol Daging Buah Burahol (Stelecocharpus burahol) Berdasarkan Cara Preparasi Simplisia. Makassar Natural Product Journal. 2023;1(3):176–84.
17. Muhtadi M, Ningrum U. Standardization of durian fruit peels (Durio zibethinus Murr.) extract and antioxidant activity using DPPH method. Pharmaciana. 2019 Nov 30;9(2):271. doi:10.12928/pharmaciana.v9i2.12652
18. Fatmawati A, Sucianingsih D, Kurniawati R, Abdurrahman M. Microscopic Identification and Determination of Total Flavonoid Content of Moringa Leaves Extract and Ethyl Acetate Fraction (Moringa oleifera L.) . Indonesian Journal of Pharmaceutical Science and Technology. 2021;1(1):66–74.
19. Herawati IE, Saepudin S. Analysis of antioxidant and standardization of ethanol extract of rumput mutiara (Oldelandia corymbosa L.). Pharmaciana. 2025 Jul 31;15(2):332–41. doi:10.12928/pharmaciana.v15i2.27939
20. Molole GJ, Gure A, Abdissa N. Determination of total phenolic content and antioxidant activity of Commiphora mollis (Oliv.) Engl. resin. BMC Chem. 2022 Dec 25;16(1):48. doi:10.1186/s13065-022-00841-x
21. Nurcholis W, Sya'bani Putri DN, Husnawati H, Aisyah SI, Priosoeryanto BP. Total flavonoid content and antioxidant activity of ethanol and ethyl acetate extracts from accessions of Amomum compactum fruits. Annals of Agricultural Sciences. 2021 Jun;66(1):58–62. doi:10.1016/j.aoas.2021.04.001
22. Khotimah TK, Krisridwany A, Orbayinah S, Harimurti S. Antibacterial Activity of Fractions from Extract Ethanolic of Hylocereus Polyrhizus Peel Against E. Coli and S. aureus.

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| | | | Journal of Fundamental and Applied Pharmaceutical Science. 2021 Apr 23;1(2). doi:10.18196/jfaps.v1i2.11001 |
| **Reviewer 3** | | | |
| 1 | Abstract<br>Page 1 | Please complete/ adjust the conclusion | Thank you for your suggestions and feedback. We've revised it by highlighting it in red.<br>The yellow root extract contained total phenolic and flavonoid contents of 87.37 mg GAE/g and 73.80 mg QE/g, respectively. At 800 ppm, the ethanolic extract and fractions, with an inhibition zone of 20.53±0.58 mm, approaching that the clindamycin phosphate (21.90±0.40 mm). Overall, these finding demonstrate that the standardized ethanolic extract of Arcangelisia flava (L.) Merr. root possesses promising antibacterial activity against P.acnes, supporting its potential as a source of bioactive compounds for further development as a natural anti-acne agent. |
| 2 | Introduction<br>Page 2 | Please complete the explanation of research gap, and the urgency of the test on P acnes | Thank you for your suggestions and feedback. We've revised it by highlighting it in red.<br><br>Previous studies have demonstrated various pharmacological properties of Arcangelisia flava (L.) Merr., including antimicrobial activity. More specifically, a previous study reported that polarity-based fractions of A. flava were able to inhibit the growth of Propionibacterium acnes. However, that study mainly focused on antibacterial screening of the fractions and did not integrate the antibacterial findings with comprehensive quality standardization of the plant material and extract, quantitative determination of total phenolic and flavonoid contents, and direct comparison between the crude extract and its fractions (6). Therefore, the relationship between the standardized quality characteristics, phytochemical content, and antibacterial activity of A. flava root extract against acne-associated bacteria remains insufficiently characterized. |

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| | | | This represents an important research gap because standardization is essential to ensure the identity, consistency, and reproducibility of medicinal plant extracts intended for further pharmaceutical development.<br>Propionibacterium acnes was selected as the test organism because it plays an important role in inflammatory acne through its interaction with the pilosebaceous unit and host inflammatory responses. Moreover, increasing resistance of P. acnes to antibiotics commonly used for acne treatment, particularly clindamycin and macrolides, has become an important therapeutic concern (7). Recent clinical studies have also demonstrated the ability of P. acnes isolates to form biofilms and have reported substantial resistance to commonly used anti-acne antibiotics (8). These problems reinforce the need to explore plant-derived antibacterial compounds as potential complementary sources of anti-acne agents.<br>The selection of A. flava is also phytochemically relevant. Recent chemical investigations have identified several alkaloidal constituents in this plant, including berberine, jatrorrhizine, and palmatine (9). Of particular interest, berberine has recently been demonstrated to exert direct antibacterial activity against P. acnes, with reported MIC and MBC values of 6.25–12.5 μg/mL and 12.5–25 μg/mL, respectively. Its antibacterial action was associated with disruption of the bacterial cell wall and membrane and alteration of genes involved in peptidoglycan synthesis (10). Accordingly, evaluation of the standardized A. flava root extract and its fractions against P. acnes provides a rational approach to link the phytochemical characteristics and quality parameters of the extract with its potential biological activity. |

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| 3 | Method<br>Extraction<br>Page 4 | The term of "stable weight" was not clear | Thank you for your suggestions and feedback. We've revised it by highlighting it in red.<br><br>Yellow root was extracted through maceration using 70% ethanol over a period of 4 x 24 hours, with the solvent being replaced every 24 hours. The extracts were concentrated using a rotary evaporator at 40–50 °C until constant weight is obtained, after which the yield was calculated |
| 4 | Result and Discussion<br>Figure 2<br>Page 11 | Please check the figure | Thank you for your suggestions and feedback.<br>There is no damage to the attached image. The difference in the image's appearance is likely due to the "Track Changes" feature in Word.<br><br> |
| 5 | Result and Discussion<br>Page 15 | Please provide the in depth result of antibacterial section | Thank you for your suggestions and feedback. We've revised it by highlighting it in red.<br>The antibacterial activity results demonstrated a clear concentration-dependent trend for the ethanolic extract and all fractions of yellow root. At 800 ppm, the ethanolic extract produced the largest inhibition zone (20.53 ± 0.58 mm), followed by the water fraction (18.60 ± 0.20 mm), ethyl acetate fraction (15.93 ± 0.35 mm), and n-hexane fraction (14.83 ± 0.50 mm). At 600 ppm, the same order of antibacterial activity was observed, with inhibition zones of 18.90 ± 0.62, 17.16 ± 0.66, 14.46 ± 0.66, and 13.26 ± |

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| | | | 0.05 mm, respectively. These findings indicate that increasing the sample concentration increased the amount of antibacterial constituents available to interact with the bacterial cells, resulting in a larger growth-inhibition zone.<br>Interestingly, the crude ethanolic extract exhibited greater antibacterial activity than each individual fraction. Although fractionation is frequently performed to enrich compounds according to their polarity, it does not necessarily result in stronger biological activity. The higher activity of the crude extract observed in this study may indicate additive or synergistic interactions among several classes of secondary metabolites that remain together in the unfractionated extract. In contrast, liquid–liquid partitioning may separate complementary bioactive compounds into different fractions or reduce the concentration of individual active constituents through incomplete recovery during the fractionation process. Therefore, the antibacterial effect of A. flava may not depend on a single fraction or compound but rather on the combined contribution of multiple constituents.<br>Among the fractions, the water fraction showed the strongest activity, reaching an inhibition zone of 18.60 ± 0.20 mm at 800 ppm. This finding suggests that relatively polar constituents may contribute substantially to the antibacterial effect of the extract. This interpretation is consistent with the phytochemical screening of the present study, which demonstrated the presence of alkaloids, phenolics, flavonoids, and saponins in the ethanolic extract. Nevertheless, because individual compounds were not isolated and quantitatively correlated with antibacterial activity in the present investigation, the activity cannot be attributed conclusively to a specific compound. |

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| | | | The antibacterial activity may partly be associated with protoberberine-type alkaloids reported in A. flava, particularly berberine (9). Recent experimental evidence demonstrated that berberine inhibited several P. acnes strains, with MIC and MBC values ranging from 6.25–12.5 µg/mL and 12.5–25 µg/mL, respectively (10). Berberine treatment caused morphological alterations of P. acnes, increased leakage of intracellular components, and affected genes involved in peptidoglycan biosynthesis, suggesting disruption of bacterial cell-wall and membrane integrity as possible antibacterial mechanisms (10). These findings provide a plausible mechanistic basis for the antibacterial activity observed for the yellow root extract, although further chemical profiling and activity-guided isolation are required to determine which constituents contribute most strongly to the observed effect.<br>The ethanolic extract at 800 ppm produced an inhibition zone of 20.53 ± 0.58 mm, which was close to that produced by the clindamycin phosphate positive control (21.90 ± 0.40 mm). However, this comparison should be interpreted cautiously because inhibition-zone diameter is influenced not only by intrinsic antibacterial potency but also by the concentration, molecular size, solubility, and diffusion characteristics of compounds in the agar medium. Therefore, the present results establish the antibacterial potential of the extract but do not demonstrate equivalence to clindamycin. Determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and identification of the major active compounds would be required to more accurately characterize its antibacterial potency and mechanism. |

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| 6 | Conclusion<br>Page 17 | Please provide sentences that meet the grammatical rules of academic writing. | Thank you for your suggestions and feedback. We've revised it by highlighting it in red.<br>This study demonstrated that yellow root extract contained a total phenolic content of 87.37 mg GAE/g and a total flavonoid content of 73.80 mg QE/g. The extract exhibited stronger antibacterial activity against *Propionibacterium acnes* than its fractions, producing an inhibition zone diameter of 20.23 ± 0.58 mm at a concentration of 800 ppm. The standardization results indicated that all evaluated parameters of the yellow root extract met the established quality requirements. The extract contained high levels of phenolic and flavonoid compounds, and its antibacterial activity was greater than that of the corresponding fractions. |