



## Flavonoid Content Combination of Averrhoa bilimbi L and Phaleria macrocarpa Fruit Extract as Chemoprevention Agent against COVID-19

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### ABSTRACT

The covid pandemic that has occurred for more than two years is still not over. Even in January 2022, with the new variant, Omicron, the number of COVID-19 cases has increased dramatically. According to statistics, on January 25 there were 3,559,000,464 new cases of COVID-19 and an average of 7 days as many as 3,397,073 cases. Therefore, it is important to take preventive measures to avoid the COVID-19 virus. One of them by eating foods high in antioxidants. Some fruits that are high in antioxidants are Averrhoa bilimbi L and Phaleria macrocarpa. Flavonoids are one of the types of antioxidants found in Averrhoa bilimbi L and Phaleria macrocarpa which function to inhibit the formation of Reactive Oxygen Species (ROS) by inhibiting enzymes in the formation of ROS and increasing the regulation and protection of antioxidants to prevent various negative impacts such as degenerative diseases and decreased immunity so that it can prevent exposure to the COVID-19 virus. The purpose of this study was to determine the levels of flavonoids in the combination of extracts of Averrhoa bilimbi L and Phaleria macrocarpa. The method used is the UV-VIS spectrophotometry method. The result of this research is that the flavonoid content of Averrhoa bilimbi L is 2.32 mg/g, the extract of Phaleria macrocarpa is 4.83 mg/g and the combination of the two is 4.77 mg/g. From these results, it can be said that the extract of Phaleria macrocarpa has the highest flavonoid content of 4.83 mg/g.

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#### Kata kunci:

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### ABSTRAK

Pandemi COVID-19 yang sudah terjadi lebih dari dua tahun masih belum berakhir bahkan pada Januari 2022 dengan adanya varian baru yaitu omicron, jumlah kasus COVID-19 meningkat drastis. Menurut statistik, pada 25 Januari terdapat 3.559.000.464 kasus baru COVID-19 dan rata-rata 7 hari sebanyak 3.397.073 kasus. Oleh karena itu pentingnya upaya pencegahan agar terhindar dari virus COVID-19. Salah satunya dengan mengonsumsi makanan tinggi antioksidan. Beberapa buah yang tinggi antioksidan adalah buah belimbing wuluh dan buah mahkota dewa. Flavonoid merupakan salah satu jenis antioksidan yang terdapat pada buah belimbing wuluh dan buah mahkota dewa yang berfungsi menghambat pembentukan Reactive Oxygen Species (ROS) dengan menghambat enzim dalam pembentukan ROS dan meningkatkan regulasi serta proteksi dari antioksidan sehingga dapat mencegah berbagai dampak negatifnya seperti penyakit degenerative dan penurunan imunitas sehingga dapat mencegah dari terpaparnya virus COVID-19. Tujuan penelitian ini yaitu untuk mengetahui kadar flavonoid pada kombinasi ekstrak buah belimbing wuluh dan buah mahkota dewa. Metode yang digunakan yaitu metode spektrofotometri UV-VIS. Hasil penelitian ini yaitu didapatkan kadar

flavonoid buah belimbing wuluh sebesar 2.32 mg/g, ekstrak buah mahkota dewa sebesar 4.83 mg/g dan kombinasi dari keduanya sebesar 4.77 mg/g. Dari hasil tersebut dapat disimpulkan bahwa ekstrak buah mahkota dewa memiliki kadar flavonoid tertinggi yaitu 4.83 mg/g.

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## INTRODUCTION

The COVID-19 pandemic is an event caused by the corona virus that can attack the human respiratory system and endanger health (Rothan & Byrareddy, 2020). COVID-19 initially appeared in Wuhan, China at the end of December 2019. For under two months, COVID-19 has caused 80,000 cases and 3,000 deaths. The rate of COVID-19 cases is still fluctuating and the virus has grown rapidly and rapidly in transmission and has given rise to several new variants such as the alpha, beta, gamma, delta variants, and the newest variant in January 2022, the Omicron variant (WHO, 2022., Awwal & Afandi, 2021). This variant has very fast transmission, thus increasing the incidence of COVID-19 cases, in January 2022 there were 3,559,000,464 new cases of COVID-19 and an average of 7 days as many as 3,397,073 cases.

To avoid infection with the corona virus, WHO (2020) recommends that you always wash your hands frequently with soap and running water, keep a distance of at least 1 meter from other people, cover your mouth and nose when coughing or sneezing, stay home when you are unwell, don't leave the house if not necessary and avoid crowds (Ilham, 2021). As it is known that the corona virus attacks the immune system so that the body is not able to deal with the virus anymore. Thus the immune system becomes the main factor in dealing with the virus (Efianita & Annisa, 2021). For this reason, efforts are needed to maintain and increase immunity, by consuming balanced nutrition, besides consuming drinks from various herbal plants can also increase body immunity. Indonesia is one of the countries that have many natural plants that can be efficacious as traditional medicine (Aminah et al., 2017). One of the plants that can be used as medicinal plants is *Averrhoa bilimbi* L and *Phaleria macrocarpa* because these plants have active substances that are beneficial to humans. In general, *Averrhoa bilimbi* L contains alkaloids, saponins, tannins, flavonoids, phenols, and triterpenoids (Hasim et al., 2019), while the *Phaleria macrocarpa* contains flavonoids, saponins, tannins, alkaloids, and phalerin (Candra, 2015). One type of active substance from the two plants that have many benefits is flavonoid compounds. Flavonoids are one of the largest natural phenol group compounds found in all green plants (Kurniawan, 2017). One of the functions of flavonoid compounds is as an antioxidant that can inhibit the formation of Reactive Oxygen Species (ROS) by inhibiting enzymes in the formation of ROS and increasing the regulation and protection of antioxidants to prevent various negative impacts such as degenerative diseases and decreased immunity (sari et al., 2019). Although there has been researching on the respective flavonoid content of the *Averrhoa bilimbi* L and *Phaleria macrocarpa*, there has been no research on the flavonoid levels of the combination of the two fruit extracts. Therefore, this study was conducted to determine the flavonoid levels of the combination of *Averrhoa bilimbi* L and *Phaleria macrocarpa* extract which is expected to work synergistically as chemoprevention against COVID-19.

## METHOD

### *Participant characteristics and research design*

This research is exploratory research conducted in a laboratory to test the levels of flavonoids of *Averrhoa bilimbi* L, *Phaleria macrocarpa*, and their combination. This research was conducted at the Integrated Research Laboratory of the Faculty of Pharmacy, Ahmad Dahlan University.

### *Sampling procedures*

### *Tools and materials*

The tools used were beaker glass, measuring flask, stirring rod, separating funnel, analytical balance, pipette, and UV-Vis spectrophotometry while the materials used were *Simplicia Averrhoa bilimbi* L, *Simplicia Phaleria macrocarpa*, quercetin solution, AlCl<sub>3</sub> solution 10% ethanol 96%, potassium acetate, and aquadest.

### *Sample preparation and extraction process*

The *simplicia Averrhoa bilimbi* L was obtained from the *Materia Medika* Laboratory of Malang and *simplicia Phaleria macrocarpa* was obtained from the Merapi Farma plantation in Yogyakarta. Then the two *simplicia* were extracted using the maceration method and maceration was performed twice to obtain a more optimal content.

### *Measurement of flavonoid levels*

Measurement of flavonoid levels using a colorimetric method that begins with the manufacture of quercetin mother liquor, 10% AlCl<sub>3</sub> solution, and potassium acetate solution. After the solution has been prepared, it is continued with the determination of the operating time by pipetting 300 L of a 1000 ppm quercetin solution and put in a 5 mL volumetric flask (concentration 60 ppm). Then added 96% ethanol, 0.5 mL of 60 ppm quercetin solution, 1.5 mL of 96% ethanol, 0.1 mL of 10% aluminum chloride (AlCl<sub>3</sub>), 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. Then measured at a wavelength of 424.2 nm for 3600 seconds. Next is the determination of the maximum wavelength by measuring the absorption in the range of 400-600 nm. After that, the sample was weighed and dissolved in 2.5 mL of 96% ethanol and sonicated for 15 minutes, then filtered. The filter results added 96% ethanol. Next, 0.5 ml of the sample solution was taken and added with 1.5 mL of 96% ethanol, 0.1 mL of 10% aluminum chloride (AlCl<sub>3</sub>), 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. Then it was incubated for 30 minutes at room temperature, and the absorbance was measured using the UV-Vis spectrophotometry method at the maximum wavelength. Samples were made in three replications for each analysis and the average value of absorbance was obtained.



Figure 1. Extraction Process

## RESULTS AND DISCUSSION

### Extraction process

*Averrhoa bilimbi L* and *Phaleria macrocarpa* were extracted using the maceration method. This extraction aims to obtain the chemical compounds contained in the two fruits, while the maceration method was chosen because the maceration method is one of the cold extraction methods to minimize damage to compounds that are not resistant to heating, especially flavonoids. In addition, the maceration method is also a simple and easy extraction method. The solvent used in this extraction is 96% ethanol solvent, the choice of ethanol solvent is based on the nature of the compound, namely flavonoids which are soluble in polar solvents such as ethanol (Zhang et al, 2018). Ethanol as a solvent also has safe properties that are not toxic, prevents the growth of mold at concentrations of more than 20%, is safe and harmless to the environment, is non-toxic, and has a low boiling point so that it is easily evaporated. These properties of ethanol make the absorption process more efficient (Putriana, 2018). The maceration results obtained are then filtrated and evaporated using a water bath to obtain a thick paste extract that can be used to test flavonoid levels.

After extraction, the two fruits were measured for flavonoid levels by colorimetric method using UV-Vis spectrophotometry which was carried out to determine how much total flavonoid content was contained in the extract of *Averrhoa Bilimbi L*, *Phaleria Macrocarpa*, or their combination.

The analysis of flavonoids was carried out using UV-Vis spectrophotometry because flavonoids contain a conjugated aromatic system so that they show strong absorption bands in the ultraviolet and visible spectral regions (Aminah et al., 2017). In this study, to determine the total flavonoid content in the sample, quercetin was used as a standard solution with a concentration series of 5, 12.5, 25, 37.5, 50, 62.5, and 75. Quercetin was chosen as the standard solution because quercetin is a flavonoid flavonol group that has a keto group at C. -4 and has a hydroxyl group on the neighboring C-3 or C-5 atoms of flavones and flavonols (Azizah & Faramayuda 2014). Measurement of the maximum wavelength absorption is carried out running from a wavelength of 400 - 600 nm. The results of running can be seen from Figure 1 which shows the maximum wavelength of quercetin standard is at a wavelength of 435 nm. Several medicinal plants containing flavonoids have been reported to have antioxidant, antibacterial, antiviral, anti-inflammatory, anti-allergic, and anti cancer activities (Ahmad et al., 2015).

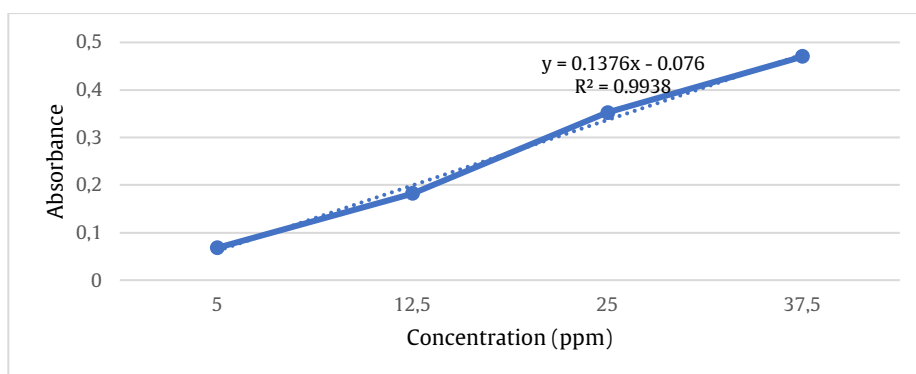


Figure 2. Quercetin Standard Solution Calibration Curve

From Figure 3, it is known that the higher the concentration used, the higher the absorbance obtained. The standard results of quercetin obtained are plotted between the content and absorbance so that a linear regression equation is obtained, namely  $y = 0.01376 x - 0.076$  with a

correlative coefficient ( $r^2$ ) value of 0.9938 which indicates this result has good validity. The quercetin calibration curve equation can be used as a comparison to determine the concentration of total flavonoid compounds in the sample extract.

**Table 1.1**  
**Flavonoid levels in each sample**

Sample Type	Replication	Concentration	Absorbance	Flavonoid Level (Mg/G)	Average Rate
<i>Averrhoa bilimbi L</i>	1	17.889	0.244	2,33	2.32
	2	17.768	0.242	2,31	
	3	17.887	0.244	2,33	
<i>Phaleria macrocarpa</i>	1	44.139	0.590	4.84	4.83
	2	44.052	0.588	4.82	
	3	44.101	0.589	4.83	
Their Combination	1	43.546	0.582	4.78	4.77
	2	43.452	0.581	4.77	
	3	43.253	0.578	4.75	

Quantitative analysis testing using UV-Vis spectrophotometry used a blank solution as control which functions as a blank (multiplying zeros) for compounds that do not need to be analyzed (Aminah et al., 2017). In the measurement of total flavonoid compounds, AlCl<sub>3</sub> was added to the sample solution which can form a complex, resulting in a shift of wavelength to the visible direction which is indicated by the solution producing a more yellow color while the addition of potassium acetate which aims to maintain the wavelength in the visible region. Incubation treatment for 1 hour before the measurement is intended so that the reaction runs perfectly so that the intensity of the resulting color is more maximal (Azizah & Faramayuda 2014).

From table 1.1 it can be seen that the flavonoid content of *Averrhoa bilimbi L* extract is 2.32 mg/g, this is in line with the research conducted by Chowdhury (2012) which stated that the *Averrhoa bilimbi L* has flavonoid compounds with levels of 2.818 mg/g. The flavonoid content of *Phaleria macrocarpa* extract is 4.83 mg/g, this is in line with research conducted by Mohamed Mahzir et al, (2018) which stated that the *Phaleria macrocarpa* has flavonoid compounds with levels of 3.22 mg/g. The extracts of *Averrhoa bilimbi L* and *Phaleria macrocarpa* fruit which have flavonoid compounds were then combined and obtained flavonoid levels of 4.77 mg/g. The higher the levels of flavonoids, the higher the benefits of flavonoids as antioxidants (sari et al., 2019). Therefore, in this study, the highest flavonoid content was in the extract of *Phaleria macrocarpa*.

The high levels of flavonoids in the extract of the *Averrhoa bilimbi L*, the *Phaleria macrocarpa*, and their combination are useful for increasing human immunity. This is because when the activity of the immune system decreases, the flavonoid content will send intracellular signals to cell receptors to increase its activity. Flavonoids as antioxidants can also stimulate the body's immune system in the form of specific antigen responses and non-specific immune responses to then produce phagocytic cells. The specific antigen response produced will cause the production of large numbers of lymphocytes, especially B lymphocytes B lymphocytes will produce antibodies which are plasma glycoproteins that will bind to antigens and stimulate the phagocytosis process (Alkandahri et al., 2018). In addition, flavonoids can also inhibit the formation of Reactive Oxygen Species (ROS) by inhibiting enzymes in the formation of ROS and increasing regulation and protection of antioxidants to prevent various negative impacts such as degenerative diseases and decreased immunity (sari et al., 2019). The flavonoids in the *Averrhoa bilimbi L* and *Phaleria macrocarpa* extract that can increase the body's immunity are the basis that the fruit extract can be a chemoprevention compound against exposure to the virus that causes COVID-19.

#### LIMITATION OF THE STUDY

The limitation of this research is that there is no research on other antioxidants besides flavonoids so it is hoped that further research can analyze all types of antioxidants found in star fruit and crown god fruit so that both fruits can be used optimally.

#### CONCLUSIONS AND SUGGESTIONS

Based on the results of the study, it can be seen that the extracts of *Averrhoa bilimbi L*, *Phaleria macrocarpa*, and the combination of the two extracts have high levels of flavonoids, namely 2.32 mg/g, 4.83 mg/g, and 4.77 mg/g, respectively. The highest flavonoid content was found in the fruit extract of *Phaleria macrocarpa*, which was 4.83 mg/g. The high levels of flavonoids are expected to be an alternative as an effort to prevent the COVID-19 virus.

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#### REFERENCES

- Alkandahri, M. Y., Buana, U., Karawang, P., Subarnas, A., Padjadjaran, U., Berbudi, A., & Padjadjaran, U. (2018). *REVIEW: AKTIVITAS IMMUNOMODULATOR TANAMAN SAMBILOTO Farmaka. September.* <https://doi.org/10.24198/jf.v16i3.14237>
- Aminah, A., Tomayahu, N., & Abidin, Z. (2017). Penetapan Kadar Flavonoid Total Ekstrak Etanol Kulit Alpukat (*Persea americana Mill.*) Dengan Metode Spektrofotometri UV-VIS. *Jurnal Fitofarmaka Indonesia*, 4(2), 226–230. <https://doi.org/10.33096/jffi.v4i2.265>
- Awwal, M. A. F., & Afandi, M. Y. (2021). the Effect of Corona Pandemic, Mine Commodity Prices and Rupiah Exchange Rate on Indonesian Sharia Share Prices in the Mining Sector 2020. *Jurnal Ekonomi Dan Bisnis Islam (Journal of Islamic Economics and Business)*, 7 (2), 350. <https://doi.org/10.20473/jebis.v7i2.29838>
- Candra, F. M. (2015). *Pemanfaatan Buah Mahkota Dewa (phaleria macrocarpha) Sebagai Green Corrosion Inhibitor Pada Baja API 5L Grade B di Lingkungan 3, 5% NACL dan H2SO4 1M.* 1–6. <http://repository.its.ac.id/id/eprint/62532>

- Efanita, K., & Sinta Nur Annisa. (2021). Membuat Minuman Sumber Antioksidan Pada Warga Rt. *Seminar Nasional Pengabdian Masyarakat LPPM UMJ*, 138, 1–10.
- Hasim, H., Arifin, Y. Y., Andrianto, D., & Faridah, D. N. (2019). Ekstrak Etanol Daun Belimbing Wuluh (*Averrhoa bilimbi*) sebagai Antioksidan dan Antiinflamasi. *Jurnal Aplikasi Teknologi Pangan*, 8(3), 86. <https://doi.org/10.17728/jatp.4201>
- Ilham, R. (2021). Correlation Between Health Protocol With COVID-19 Cases In Workers In dr. Zainal Umar Sidiki Hospital. *Jurnal Aisyah: Jurnal Ilmu Kesehatan*, 6, 89–92. <https://doi.org/10.30604/jika.v6is1.767>
- Kurniawan, D. (2017). *Universitas Negeri Semarang Semarang 2017. 0024078603*, 1–52.
- Putriana, A. (2018). Ekstrak buah belimbing wuluh (*Averrhoa bilimbi* L) Sebagai Ovisida Keong Mas (*Pomacea canaliculata* L.). *Universitas Negeri Raden Intan. Lampung*, 1–126.
- Rothan, H. A., & Byrareddy, S. N. (2020). *Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID- 19 . The COVID-19 resource centre is hosted on Elsevier Connect , the company ' s public news and information . January.*
- sari, A. K., Ayuchecaria, N., & Febrianti, D. R. (2019). Analisis Kuantitatif Kadar Flavonoid Ekstrak Etanol Daun Belimbing Wuluh (*Averrhoa bilimbi* L.) di Banjarmasin dengan Metode Spektrofotometri UV-VISIBLE. *Jurnal Insan Farmasi Indonesia*, 2(1), 7–17. <https://doi.org/10.36387/jifi.v2i1.315>

