

# Effect of Solvent Ratio of *Phaleria macrocarpa* Fruit Extract on Yield, Chemical Content and its Antimicrobial Activity.

Najma Afrahan Nufus<sup>1</sup>, Eko Widodo<sup>2\*</sup>, and Heli Tistiana<sup>2</sup>

<sup>1</sup> Master Student of the Faculty of Animal Science, Brawijaya University, Malang 65145, Indonesia

<sup>2</sup> Lecturers of the Faculty of Animal Science, Brawijaya University, Malang 65145, Indonesia

**Abstract.** The research aimed to determine the effect of solvent ratio of *Phaleria macrocarpa* fruit on the extracted yield, chemical contents and antimicrobial activity for *Escherichia coli* and *Salmonella sp.* The materials used were *Phaleria macrocarpa* fruit and aquadest. The extraction method used in this study was Ultrasound-Assisted Hydro-distillation Extraction (UAHDE). The antimicrobial activity was detected by the inhibition zone of *Phaleria macrocarpa* fruit extract against Lactic Acid Bacteria, *Escherichia coli* and *Salmonella sp.* using the well diffusion method. The results showed that with an extract ratio of 1:2 produced a yield of (87.3 ml), 1:4 (362 ml), and 1:6 (486 ml). The results of the chemical test of *Phaleria macrocarpa* showed that extract ratio 1:2 (phenol 314.64 mg GAE/100g and flavonoid 39.89 mg EQ/100g), 1:4 (phenol 228.02 mg GAE/100g and flavonoid 21.61 mg EQ/100g), 1:6 (phenol 150.89 mg GAE/100g and flavonoid 15.35 mg EQ/100g). Comparison of the ratios of 1:4 and 1:6 showed significant results ( $P<0.05$ ) inhibition of *Salmonella sp.* bacteria with an inhibition zone of  $1.1 \pm 0.2\text{mm}$  and *Escherichia coli* of  $1 \pm 0.0\text{mm}$ , while no inhibition was detected for Lactic Acid Bacteria. It can be concluded that the extract of the *Phaleria macrocarpa* fruit have the potential to be used as a feed additive for poultry.

**Keywords:** antimicrobial activity, *Phaleria macrocarpa*, poultry feed additive, solvent ratio.

## 1 Introduction

Feed is a key component in poultry production systems because it directly impacts poultry growth, health, and productivity. Feed quality is determined by its nutritional content, including protein, fat, vitamins, and minerals, which must be tailored to the physiological needs of poultry at each growth stage. The problem often faced by poultry farmers include high and fluctuating feed prices and limited availability of quality feed ingredients. This situation encourages some farmers to use alternative feed ingredients, which can impact poultry production performance and health. Farmers often use antibiotics to improve poultry health, growth potential, and productivity [1]. Inappropriate and excessive use of antibiotics in poultry feed can have negative impacts, such as the emergence of antibiotic-resistant

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\* Corresponding author: [eko.widodo@ub.ac.id](mailto:eko.widodo@ub.ac.id)

bacteria and increased levels of antibiotic residues in animal products. The Indonesian government has banned the use of Antibiotic Growth Promotor (AGP). The ban on antibiotic use has prompted the need to find safe and effective alternatives to antibiotics. Feed additives have the potential to replace antibiotics. Feed additives can be classified into two types: nutritive feed additives such as vitamins and minerals, and non-nutritive feed additives such as acidifiers, probiotics, prebiotics, enzymes, and phytobiotics. Phytobiotics are plant-based feed additives containing biologically active substances that provide multiple physiological benefits to poultry. Their use has been widely studied as a natural alternative to AGP [2]. Phytobiotics are known to possess antimicrobial, immunomodulatory, antifungal, and anti-inflammatory activities, which contribute to improved growth performance, animal health, and the quality of poultry products [2]. The bioactive compounds in phytobiotics do not leave residues in meat, making them safe for human consumption without causing side effects. Several bioactive plant compound that can be used as feed additive including phenol, saponins, tannin, and flavonoid [3].

A plant that has the potential containing high flavonoid when being extracted is *P. macrocarpa* fruit. According to the Indonesian Central Bureau of Statistics, the fruit production reached in 2024 was 3,498,168 kg. The fruit is known as a medicinal plant, a type of plant that grow all year round. *P. macrocarpa* fruit contains bioactive compounds including flavonoids, phenols, tannins, and saponins [4 ; 5]. These bioactive compounds have been reported to have antimicrobial properties and improve health, so that potential for application in functional feed or natural antimicrobials. This study aims to evaluate the total yield based on the solvent ratio used, active substances contents and the bacterial inhibition of *P. macrocarpa* fruit extract as an effective and innovative poultry feed additive. The results of this study are expected to provide benefits and solutions for farmers regarding the impact of natural antibiotics on poultry health and productivity

## 2 Material and methods

### 2.1 Plant preparation

Fresh fruit of *P. macrocarpa* was purchase online. Based on the seller's description, the fruits were harvested from plants cultivated in West Bandung Regency, Indonesia. The parts of fruit used are the pericarp, mesocarp, and endocarp. The fruit is washed, then the seeds are separated. The next step was to chop it into small pieces, then the fruit is ready to be used for extraction.

### 2.2 Extraction methods

This extraction uses the Ultrasound Assisted Hydro-distillation (UAHDE) method using distilled water as the solvent. Each solvent ratio (1:2, 1:4, and 1:6) was mixed with distilled water. The mixture is stirred and put into the EECOO Digital Ultrasound Cleanser Sonicator with a frequency of 40kHz for 20 minutes and continued with Hydro-distillation with periodic temperature checks, the optimum steam temperature for distillation was 93°C. The distillation process was carried out for 60 minutes. After that, the extract was filtered, then the results were taken for phytochemical tests and bacterial inhibition tests.

The yield extract results are calculated for total yield using the formula:

$$\text{yield \%} = \frac{\text{the extract obtained (ml)}}{\text{weight of fresh mahkota dewa fruit (g)}} \times 100 \quad (1)$$

### 2.3 Phenolic content test

Phenol analysis was performed at each solvent ratio (1:2, 1:4, and 1:6). Phenolic content was determined using the Folin–Ciocalteu method with gallic acid as the reference standard. Gallic acid standard solutions were prepared at concentrations of 50, 100, 150, and 200 ppm. An aliquot of 0.5 mL of each standard solution was reacted with 5 mL of Folin–Ciocalteu reagent followed by the addition of 4 mL of 7.5% Na<sub>2</sub>CO<sub>3</sub> solution. The mixture was incubated at room temperature for 60 minutes, and the absorbance was measured at 743 nm using a UV–Vis spectrophotometer. A calibration curve was constructed based on gallic acid concentration and absorbance, and the resulting regression equation was used to calculate phenolic content of samples.

### 2.4 Flavonoid content test

Flavonoid content was determined using the aluminium chloride (AlCl<sub>3</sub>) colorimetric method with quercetin as the standard. Quercetin standard solutions were prepared at concentrations of 20, 40, 60, 80, and 100 ppm. An aliquot of 1 mL of each standard solution was reacted with 1 mL of 2% AlCl<sub>3</sub> solution, followed by the addition of 8 mL of 5% acetic acid. The mixture was incubated at room temperature for 30 minutes, after which the absorbance was measured at 410 nm using a UV–Vis spectrophotometer. A calibration curve was constructed based on the relationship between quercetin concentration and absorbance, and the resulting regression equation was used to calculate flavonoid content of samples.

### 2.5 Inhibition zone test

The inhibition zone analysis of *P. macrocarpa* extract bacteria was carried out using the well diffusion method. First, a suspension of Lactic Acid Bacteria, *Escherichia coli* and *Salmonella sp.* was prepared with a density of approximately 10<sup>6</sup> CFU/mL along with 11.2 g of Nutrient Agar media and dissolved in 400 mL aquadest, heated using a hot plate magnetic stirrer until the solution was homogenous. Then sterilized at 121°C for 1 hour using an autoclave. The sterile media was poured into sterile petri dishes, each with 20 mL and left to solidify at room temperature. After the media had hardened, 1,000 µL of bacterial suspension was inoculated onto the agar surface using the spread plate method with a sterile glass rod until evenly distributed. Four holes with a diameter of 6.5 mm were then made in each petri dish using a sterile cork borer. Each hole was filled with 1 mL of *P. macrocarpa* fruit extract according to the solvent ratio treatment (1:2, 1:4, and 1:6), with concentrations of 50 and 100%. Each treatment was given a positive control of 2% chloramphenicol and aquadest as a comparison.

The treated petri dishes were covered and covered with plastic wrap and incubated at 37°C for 24 hours. After the incubation period, observations were made by observing the zone of inhibition formed around the wells to indicate bacterial growth inhibition by the extract. The diameter of the inhibition zone was measured vertically and horizontally using a vernier calliper, then the results were averaged to determine the strength of bacterial inhibition. The formula for bacterial inhibition is:

$$\text{Inhibition zone} = \frac{d_1 + d_2}{2} - x \quad (2)$$

Description:

d1 = vertical diameter of the clear zone in the media

d2 = horizontal diameter of the clear zone in the media

x = well hole

## 2.6 Data analysis

Chemical content and yield in this study were analyzed descriptively. Results are presented as averages, which were then interpreted to determine the potency of tested samples. Inhibition zone results from each treatment were tabulated and analyzed by using Completely Randomized Design (CRD) and ANNOVA using SPSS version 22 software and in case of significant difference continued with Duncan's Multiple Range Test.

## 3 Results and Discussion

### 3.1 Yield percentage

The results of the yield percentages shown in Table 1.

**Table 1.** Yield percentages *P. macrocarpa* fruit extract

Ratio	Extract result (ml)	Fresh fruit weight (g)	Yield percentage (%)
1:2	131	150	87.33
1:4	543	150	362
1:6	730	150	486.6

As shown in Table 1, a solvent ratio of 1:6 produced the highest total yield. The extract yield exceeding 100% is caused by the water remaining in the extract and other hydrophilic compounds due to the use of aquadest as a solvent and extraction temperature below the boiling point of water (100°C), this condition produces an extract of more than 100%. This research use Ultrasound Assisted Hydro-distillation (UAHD) method using ultrasonic waves which can significantly reduce extraction time and increase yield by creating cavitation bubbles in the solvent, which facilitates the release of target compounds from plant cell walls [6]. The extraction yield of the active components from plant materials is well-known to be affected by the ratio of solvent to raw material, which is an important factor.

Phenolic compounds such as phenols and flavonoids are greatly influenced by the type of solvent used in the extraction process. The yield containing phenolic compounds depends on the type of solvent used, so it is necessary to select solvents based on their polarity and safety [7]. Solvents with polar properties are the best solvents for extracting phenolic components because of the ability to dissolve polyphenol compounds. Polar solvents can improve the quality and yield of the resulting yield [8]. The total yield is influenced by the polarity level of the organic solvent used in the extraction process, resulting in affecting the type and quantity of compounds extracted [8]. In the hydrodistillation method, water not only serves as a heating medium but also as an effective solvent for dissolving phenolic and flavonoid compounds. During heating, plant tissue is damaged allowing phenolic compounds to be released and dissolved in water more easily. Therefore, hydrodistillation using distilled water as a solvent can be used to obtain phenolic and flavonoid compounds [9].

### 3.2 Phenol and flavonoid content

The results of the phenolic and flavonoid contents shown in Table 2.

**Table 2.** Phenolic and flavonoid contents of *P. macrocarpa* fruit extract

Ratio	Phenol Content (mg GAE/100g)	Flavonoid Content (mg EQ/100g)
1:2	314.64	39.89
1:4	228.02	21.61
1:6	150.89	15.35

The ratio of *P. macrocarpa* fruit to solvent is a critical factor affecting the extraction efficiency of bioactive compounds, including phenolics and flavonoids. In this study, extraction ratios of 1:2, 1:4, and 1:6 were applied; however, the highest phenolic and flavonoid contents were observed at a ratio of 1:2. Based on Table 2, the highest phenolic and flavonoid content is shown at a ratio of 1:2. A higher solvent ratio results in a higher concentration, accelerating the transfer of phenolic compounds from the material into the solvent. This results in a more thicker extract. Conversely, using an excess of solvent lowers the concentration and causes the extract to become more dilute, so increasing solvent volume does not always lead to increased yield after a certain point is reached [10]. So, the higher the solvent ratio, the lower the total phenolic or yield obtained.

### 3.3 Inhibition zone test

The results of the inhibition zone are shown in Table 3

**Table 3.** Inhibition zone of *P. macrocarpa* fruit extract ratio 1 :2

Treatment	Inhibition zone (mm)		
	LAB	<i>Escherichia coli</i>	<i>Salmonella sp.</i>
P0	0	0	0
P0+	29.25±10.4	10.7±2.6	11.7±5.9
P1	0	0	1.1±0.2
P2	0	1±0.0	0

The table shows that the *P. macrocarpa* fruit extract did not inhibit Lactic Acid Bacteria (LAB). LAB are known to improve the composition of the gut microbiota, so improving the overall microbial balance in poultry. This suggests that the use of *Lactobacillus* acts as an effective alternative to Antibiotic Growth Promoters (AGP) by improving growth performance and maintaining gut health in poultry populations from *Salmonella sp.* Increasing the beneficial microbial community in the digestive tract, LAB can help suppress the growth of pathogenic bacteria, so that it can improve poultry health.

The addition of *P. macrocarpa* fruit extract at a concentration of 50% resulted in a 1.1 ± 0.2 mm if tested against *Salmonella sp.* Gram-positive bacteria are more affected than Gram-negative bacteria, possibly due to the permeability barrier of the outer membrane of Gram-negative bacteria, which makes them more resistant to antimicrobial agents. This may be due to the presence of flavonoid compounds in *P. macrocarpa* extract [11]. The extract ratio was 1:2, containing phenolic compounds (314.64 mg GAE/100g) and flavonoid compounds (39.89 mg EQ/100g). The inhibitory effect on *Salmonella sp.* bacteria in this study was relatively weak, making it ineffective when compared to 2% chloramphenicol. The diameter of the *Salmonella sp.* inhibition zone produced in treatments P1 and P2 was not significantly different, while P1 and P0+ were significantly different.

*P. macrocarpa* fruit extract at a concentration of 100% also inhibited *Escherichia coli* bacteria by 0.5 ± 0.0 mm. The different inhibitory effects observed at 50% and 100%

concentrations indicate that the antibacterial activity of phenol and flavonoid is not always concentration-dependent, but rather influenced by diffusion ability and bacterial sensitivity. The well diffusion method concentration 50% allows better diffusion of bioactive compounds into the agar medium, result in inhibition of *Escherichia coli* growth. In contrast, the 100% extract is too concentrated, limiting diffusion and preventing the formation of an inhibition zone. Previous research explains that *P. macrocarpa* fruit extract is able to inhibit *Escherichia coli* bacteria with a higher bacterial inhibition rate than in this study, namely 2.17 mm in the pericarp and 1.47 mm in the mesocarp and seeds [5]. The diameter of the inhibition zone in treatments P1 and P2 was significantly greater than in P0+. This can occur because *Escherichia coli* is resistant to antimicrobials when compared to *Salmonella sp.*, *Escherichia coli* is able to produce enzymes that can decompose antimicrobial activity, changes in cell membrane permeability, and increases in endogenous substances that act antagonistically to antimicrobial activity. In some treatments, the inhibition zone against *Escherichia coli* was smaller than *Salmonella*, indicating that *Escherichia coli* showed higher tolerance to the extract under certain conditions [12]. *Escherichia coli* is also able to change the structure of enzymes or bacterial membranes so that it can withstand antimicrobial substances.

## 4 Conclusion

*P. macrocarpa* fruit extract contains phenols and flavonoids, making it an antimicrobial agent. Although the highest inhibitory effect on *Salmonella sp.*, and *Escherichia coli* was achieved with 2% chloramphenicol, the rules and side effects of adding antibiotics to poultry feed must be considered and adjusted accordingly. Farmers may consider using *P. macrocarpa* fruit extract as a substitute for Antibiotic Growth Promotor (AGP).

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