

PHYTOCHEMICAL COMPOUND LEVELS IN BAMA LEAF EXTRACT AND ITS INHIBITORY ACTIVITY AGAINST *Salmonella Thypimurium* ATCC 19430

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Abstract

Background: Diarrhea is one of the intestinal infection symptoms that can cause poor absorption to loss of nutrition if not treated immediately. *Salmonella* spp. is 1 of the 4 fundamental global causes of diarrhea diseases and antibiotics have been widely used to treat infections caused by this group of bacteria. WHO reported that the antibiotic resistance of *Salmonella* spp. has increased in past years. For those reasons, efforts have been made to discover new antimicrobial compounds from various sources, including the ethanol extracts of Bama leaves. **Aims:** to determine the phytochemical compounds (flavonoids, tannins, and phenols) in the ethanol extract of Bama leaves and their inhibitory effect against the bacteria *Salmonella typhimurium* which causes diarrhea. **Methods:** Design method used is RAL 5 x 4, 5 treatments were concentration of Bama leaf extract 6,25%, 12,5%, 25%, 50% and 75% and 4 replicates for each test. **The Result:** The phytochemical quantitative analysis showed bama leaf extract had 520,99 mgQE flavonoid / 100 gram, 1465,19 mgTAE tannin/100 g and 16531,78 phenol/100 g extract. **Conclusion:** All contrentation of bama leaf extract could not inhibit the growth of *Salmonella thypimurium* ATCC 19430

Keywords: Bama leaf extract, phytochemical, *Salmonella thypimurium*, Sensitivity Test

1. Introduction

Stunting is the impaired growth and development that children experience from poor nutrition, repeated infection and inadequate psychosocial stimulation. Diarrhea is one of the infectious diseases that can lead to disturbances in nutrient absorption and the loss of essential nutrients if not treated immediately. According to data from the Indonesian Ministry of Health, the prevalence of diarrhea in 2018 was approximately

37.88%, equivalent to around 1,516,438 cases in toddlers. This prevalence increased in 2019 to 40%, or approximately 1,591,944 cases in toddlers (1).

In Clinically, the causes of diarrhea can be categorized into six major groups such as infection, malabsorption, allergies, poisoning, immunodeficiency, and other factors. Cases of diarrhea in Indonesia are typically caused by bacteria such as *Vibrio cholerae*, *Shigella* spp, *Escherichia coli*,

Salmonella spp, and *Campylobacter jejuni*

(2). Among these, *Salmonella* is the most common bacterial group responsible for food poisoning. *Salmonella* contamination is usually the result of poor hygiene and improper food handling practices (3).

Antibiotics have been widely used in the treatment of intestinal infections. Excessive use of antibiotic can pose risks such as bacterial resistance, the retention of toxic substances, and antibiotic residues (4). This has triggered a trend in society to back to nature in the field of healthcare, because does not side effect likes antibiotics (5).

Indonesia as a tropical country, has a wealth of flora that can be utilized as medicine. One of these is the Bama plant (*Plumbago zeylanica* L.). The leaves and roots are efficacious as medicine for various diseases, including the use of leaves for treating arthritis or rheumatism, relieving flatulence, urinary difficulties, headaches, alleviating joint pain, skin irritations, stomachaches, regulating menstruation, and urinary tract infections. Bama leaves are also known to be beneficial for digestion.

The study analyzed Bama leaf extract and obtained compound of alkaloids, saponins, tannins and glycosides (6). The

effect of crude extract of bama leaves in inhibiting *E.coli*, *Bacillus cereus*, *Staphylococcus aureus* and *Candida* had been observed and discovered that crude extract of bama leaves could inhibit and had potential antimicrobial activity (7). Research conducted by Dhale and Markandeya, revealed that Bama leaf extract has antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *E. coli*, and *Pseudomonas aeruginosa*. The solvents were used such as ether, chloroform, and alcohol (8). Therefore, this study aims to determine the effect of ethanol extract of Bama leaves in inhibiting *Salmonella typhimurium* which cause diarrhea.

2. Method

The type of this study was true experimental study with a posttest-only control design. The first group to be treated was called the experimental group, consisting of Bama leaf ethanol extract with concentrations of 6.25%, 12.5%, 25%, 50%, and 75%. The second group was a control group consisting of a positive control which is an antibiotic that is commonly used to treat Salmonellosis, namely chloramphenicol and a negative control which is used as a working control. Each treatment was repeated four times

according to the Federer Formula calculations.

Salmonella typhimurium ATCC 19430 was obtained from the Microbiology Laboratory at Faculty of Medicine, Udayana University. Meanwhile Bama leaves were obtained from Nusa Penida Island, Klungkung. The Bama leaves were extracted at the Post-Harvest Medicinal Plant Processing Center (P4TO) in Karangasem. Quantitative phytochemical analysis was conducted in the Agriculture Laboratory and the sensitivity test was carried out in the Microbiology Laboratory at Warmadewa University.

Bama leaves had been picked, cleaned, and dried were pulverized into fine powder (simplicia). Subsequently, the Bama leaf powder was macerated using 96% ethanol for six days. The filtrate from the Bama leaves was collected, and the extraction process was carried out using an evaporator until a crude extract was obtained. Crude extract of Bama leaf were going to be utilized for quantitative phytochemical analysis (phenols, flavonoids, and tannins) and for testing its inhibitory activity against *Salmonella typhimurium* ATCC 19430.

The determination of total phenols was carried out using the *Folin-Ciocalteu*

method. About 0.01 g of the crude extract was diluted in 5 ml of citrate phosphate buffer according to the treatment. A sample of 0.1 ml was pipetted and mixed with 0.3 ml of 70% ethanol. Subsequently, 0.4 ml of *Folinciaocalteau* reagent was added and the mixture was incubated for 6 minutes. After the incubation process, 4.2 ml of 5% Na_2CO_3 was added, followed by vortexing and then incubated for 90 minutes. The absorbance was read at a wavelength of 760 nm. The reading results were compared to a standard curve constructed using gallic acid.

The determination of total flavonoids was conducted using a spectrophotometer following the AlCl_3 method. About 0.01 g of the extract was diluted in 5 ml of citrate phosphate buffer according to the treatment. Then, 1 ml of the sample was mixed with 4 ml of distilled water and 0.3 ml of NaNO_2 10% solution was added. The mixture was incubated for 5 minutes, and then 0.3 ml of AlCl_3 10% solution and 2 ml of NaOH 1% solution were added. The sample was immediately tested using a spectrophotometer at a wavelength of 510 nm.

The determination of total tannin extract was analyzed using the *Folin-Denis* method. A total of 0.01 g of the extract

was diluted in 5 ml of citrate phosphate buffer according to the treatment. The diluted sample was pipetted in 0.25 ml, and added 0.25 ml of *Folin-Denis* reagent. The mixture was vortexed and added 2 ml of Na₂CO₃ 5%. The solution was vortexed again and incubated for 30 minutes. The absorbance was measured using a spectrophotometer at a wavelength of 725 nm.

The media used to observe the bacterial growth inhibition zones is *Mueller Hinton Agar* (MHA). A *Salmonella typhimurium* bacterial suspension is prepared by adding 1-3 pure culture colonies into a tube containing 5 ml NaCl 0,9%. It is then compared to the standard of turbidity at 0.5 McFarland.

The antibacterial activity test was conducted using the disk diffusion method. Empty disk-shaped filters were saturated with bama leaf extract at various concentrations : 6.25%, 12.5%, 25%, 50%, and 75%. The negative control utilized sterile distilled water and the positive control used Chloramphenicol antibiotic disks. The bacterial suspension was inoculated onto the surface of MHA media. The negative and were then placed on the MHA media. The negative and positive control were placed on separate

MHA media. This procedure was repeated four times. The media with the disk filters were incubated at 37°C for 24 hours in an inverted position. The results were reported by measuring the diameter of the inhibition zones formed using a caliper. The obtained inhibition zone diameters were analyzed using statistical tests to assess differences among the concentration treatments.

3. Result and Discussion

This study utilized Bama leaves (*Plumbago zeylanica* L.) which are commonly found in Nusa Penida, Klungkung. Bama plants typically grow as shrubs. A crude extract was obtained through the maceration and extraction process. The maceration method dissolves active compounds due to the difference in concentration between the active compounds inside and outside the cell causing the active compounds to exit until the concentration equilibrium is reached. The maceration process uses 96% ethanol because ethanol is more selective and mold is difficult to grow at ethanol concentrations of 20% and above and non-toxic. Ethanol is semi-polar so it can dissolve active ingredients in plants that are polar, semi-polar, and non-polar.

The quantitative phytochemical analysis aims to determine the total content of secondary metabolites present in simplicia or crude extract. The total concentration of phytochemical compounds (flavonoids, tannins, and phenols) in the ethanol extract of Bama leaves iare presented in the table below.

Table 1. Total Content of Phytochemical Compounds in the Ethanol Extract of Bama Leaves

No	Replication	Flavonoid Test (mgQE/100g)	Tannin Test (mgTAE/100g)	Phenol Test (mgGAE/100g)
1	1	493,42	1475,41	16606,17
2	2	474,33	1443,21	16492,20
3	3	595,24	1476,97	16496,99
Average		520,99	1465,19	16531,78

Based on table 1, the total flavonoid concentration in the ethanol extract of Bama leaves is 520.99 mgQE/100g. The total flavonoid concentration obtained can be compared with other plants, such as soursop leaves (*Annona muricata* L.), which have a total flavonoid concentration of 2.82% (282 mg/100g) (9). However, the total flavonoid concentration in mulberry leaves (*Muntingia calabura* L.) is 13.375 mgQE/100g (10). Therefore, it can be concluded that the total flavonoid concentration in Bama leaves is higher than that in soursop and mulberry leaves.

Flavonoids have various pharmacological activities, namely as steroid-genesis modulators, neuroprotective agents, anti-inflammatory substances,

immunoregulators, antibacterial, anticancer, antidiabetic agents, antioxidants, antiviral, estrogenic agents, and playing a role in neurodegenerative diseases. They also have function as inhibitors of AChE and BChE and have hepatoprotective (11). Flavonoids act as antibacterial agents through several mechanisms, including inhibiting nucleic acid synthesis, hindering cytoplasmic membrane function, and disrupting bacterial energy metabolism. Based on the study results of Manik, Hertiani, and Anshory, the antibacterial activity of ethanol extract from mulberry leaves and each fractions was 93% influenced by the total flavonoid concentration (12).

Based on table 1, the total tannin concentration in the ethanol extract of

Bama leaves is 1465.19 mgTAE/100g. The tannin concentration in soursop leaf extract is 161.53 mg/100g (13). However the tannin concentration in mulberry leaf extract is 137.15 mg GAE/100g extract (10). Therefore, it can be concluded that the tannin concentration in Bama leaves is higher than soursop and mulberry leaves. Tannin is one of the active secondary metabolites with several properties such as being an astringent, anti-diarrheal, antibacterial, and antioxidant. The mechanism action of tannins as antibacterials is by inhibiting reverse transcriptase and DNA topoisomerase enzymes, preventing the formation of bacterial cells. This is achieved by its ability to inactivate microbial cell adhesins, enzymes, and disrupt transport proteins in the inner cell layer (14).

Based on table 1, the total phenol concentration in the ethanol extract of Bama leaves is 16,531.78 mgGAE/100g. The total phenol concentration in soursop leaf extract is 27.14 µg GAE/g, which is equivalent to 2.714 mg GAE/100g¹³. The total phenol concentration in mulberry leaf extract is 22,389 mgGAE/100g¹⁰. Therefore, it can be concluded that the total phenol concentration in Bama leaves is

higher than in soursop leaves but lower than in mulberry leaves.

The antibacterial mechanism of phenolic compounds involves interfering with the peptidoglycan components of the cell wall of Gram-positive bacteria. This interference prevents the binding of N-acetylmuramic acid to the muropeptide structure, which is responsible for providing rigidity to the cell wall. As a result, bacterial cell wall synthesis is disrupted and does not form correctly. This leads to the loss of the rigid cell wall in bacteria, leaving the cell membrane vulnerable to damage and leakage. Positive correlation analysis with a correlation coefficient of 0.577 indicates that as the antibacterial activity increases, the phenol content in the *S. muticum* simplicia also increases (15).

The observation of antimicrobial activity was conducted by measuring the diameter of the inhibition zone around the disks containing the extract. Based on figure 1, ethanol extracts of Bama leaves at concentrations of 6.25%, 12.5%, 25%, 50%, and 75% have not able to inhibit the growth of *Salmonella typhimurium* ATCC 19430 bacteria.

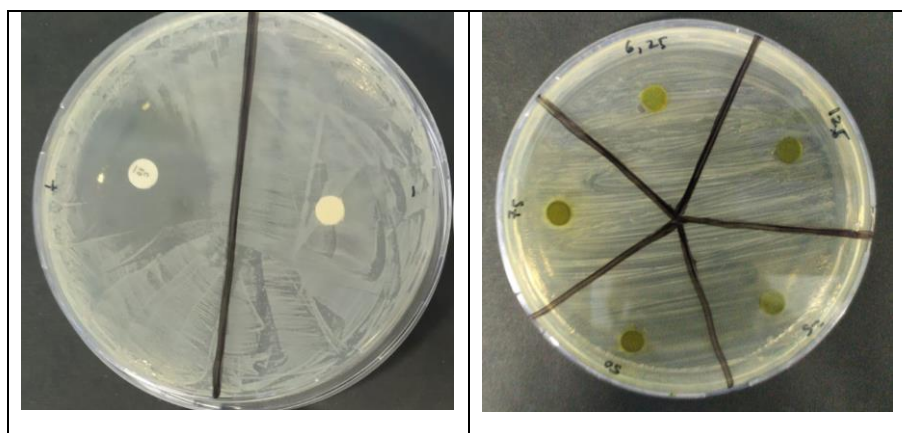


Figure 1. Inhibition zones of chloramphenicol, negative control, and ethanol extracts of Bama leaves at concentrations of 6.25%, 12.5%, 25%, 50%, and 70%.

Several factors that influence antibacterial activity include the type of bacteria being inhibited, the content of antibacterial compounds, the concentration of the extract, and the diffusion capacity of an extract. Furthermore, variations in cell wall structure also determine the activity, penetration, and binding of antibacterial compounds. *Salmonella typhimurium* is a Gram-negative bacterium with an outer membrane composed of three layers, including lipopolysaccharides, lipoproteins, and phospholipids, with porins formed from proteins. This outer membrane acts as a barrier against antibiotics, digestive enzymes, and dry conditions. This aligns with the study findings of Jamili, Hidayat, and Hifizah which showed that Gram-positive bacteria, such as *Staphylococcus aureus* produced larger inhibition zones compared to Gram-negative bacteria like

Salmonella typhi (16). This is because of the differences in the cell wall structure between gram-positive and gram-negative bacteria.

The phytochemical compounds in the ethanol extract of Bama leaves, including flavonoids, tannins, and phenols have antibacterial properties but have not been able to inhibit the growth of *Salmonella typhimurium*. This may be due to the structure of the *Salmonella typhimurium* bacterial cell membrane which consists of three layers. Therefore, active ingredients have difficulty penetrating the cell. The study results of Lio, Useng, and Ramang indicate that a 60% concentration of red spinach leaf extract exhibits resistance to *Salmonella typhimurium* (17). This suggests that the cell wall of *Salmonella typhimurium* is indeed more

challenging to penetrate for active ingredients in the extract.

4. Conclusion

Based on this study, it can be concluded that the ethanol extract of Bama leaves (*Plumbago zeylanica* L.) has a total flavonoid content of 520.99 mgQE/100g, a total tannin content of 1465.19 mgTAE/100g, and a total phenol content of 16531.78 mgGAE/100g. However, the ethanol extract of Bama leaves has not been able to inhibit *Salmonella typhimurium* ATCC 19430.

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