

## **Antibacterial Activity Test of Ethanol Extract and SAP of Betung Bamboo Shoot (*Dendrocalamus asper*) Against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* Bacteria**

**Anif Nur Artanti\* and Farikatul Sufi Mujahidah**

Department of Pharmacy, Faculty of Mathematics and Natural Science, Universitas Sebelas Maret, Jl. Ir. Sutami  
 36A Kentingan Jebres Surakarta 57126, Indonesia

\*Corresponding author: [antantinuranif@gmail.com](mailto:antantinuranif@gmail.com)

### **Abstract**

Boiled water from bamboo shoots is often used for pneumonia and a cleanser for the wound. It is known that bamboo can be an antibacterial agent because it has saponin, alkaloid, and flavonoid. This research aims to study whether ethanol extract and sap from bamboo betung shoot (*Dendrocalamus asper*) has secondary metabolism and antibacterial activity against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The extract was taken by maceration method using ethanol 96%, and the sap was taken from the water of bamboo shoot. Antibacterial activity test was conducted using disc diffusion method to measure the magnitude of inhibitory power at six concentration ratios of b/v solutions (10%; 20%; 30%; 40%; 50%; 60%). The result showed that ethanol extract and sap of bamboo betung shoot contained saponin, quercetin, and quinine which were supposed to have antibacterial activity against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The largest inhibitory zone diameter was found in ethanol extract 60% with the category of moderate inhibition of  $9.05 \pm 0.12$  mm against *Klebsiella pneumoniae* and  $5.07 \pm 0.13$  mm against *Pseudomonas aeruginosa*. While the sap with a concentration of 60% was included in the weak inhibitory category, which was  $5.65 \pm 0.05$  mm in the *Klebsiella pneumoniae* and  $4.81 \pm 0.22$  mm in *Pseudomonas aeruginosa*.

**Keywords:** antibacterial; bamboo shoot; betung bamboo.

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### **Introduction**

*Klebsiella pneumoniae* is a gram-negative opportunistic pathogenic bacteria that can cause respiratory infections, urinary tract infections, nosocomial infections, and even death by up to 10% in humans (1). *Pseudomonas aeruginosa* is a gram-negative bacteria that can cause infections in wounds and burns that cause bluish-green pus, urinary tract infections, respiratory infections that cause pneumonia, mild external muscularity in swimmers, and eye infections (2). Both of these bacteria can be found in most human environments (3).

The boiled water from bamboo shoots is used to treat coughing up blood (pneumonia) and is used as a wound cleanser (4). Bamboo

shoots have excellent antioxidant, anti-free-radical, and anti-aging effects because of flavones and glycosides (5). Betung bamboo leaves (*Dendrocalamus asper*) have antibacterial activity against *E. coli* bacteria. All bacteria and fungi grow well in wet media and humid air. Water has a vital role in life because microorganisms can only take food from outside into the solution (holophytic). Bamboo shoots are used as an infection treatment, but bamboo shoots' water content is relatively high. Every 100 grams of fresh bamboo shoots has water content of 91% (6). The water content of the *Schizostachyum brachycladum* Kurz bamboo shoot ethanol extract was 9,12% (7). So far, there has been no test for the antibacterial activity of bamboo

shoots against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

### Material and Methods

This study was taken on Pharmacy Laboratorium and FMIPA Intergrated Laboratory. In this study, the extract was taken by the maceration method using 96% ethanol, and the sap was taken from the juice of bamboo shoots. Qualitative testing of the chemical content of bamboo betung using thin layer chromatography and tube testing. The antibacterial activity test used the disc diffusion method to measure the amount of inhibition at six concentration ratios of the w/v solution (10%;20%;30%;40%;50%;60%).

The tools used in this study include a set of maceration tools, blender, flannelette, stirring rod, rotary evaporator (Yamato RE-301-AW), UV light 254 nm, test tube (Pyrex), test tube rack, bunsen, sterile cotton swab, petri dish, measuring cup (Pyrex) 25 ml, ose needle, 10-100  $\mu$ l series micropipettes (Master ptt®), bunsen, yellow tip, tweezers, analyte scale, Moisture Analyzer (Ohaus MB23), Laminar Air Flow (Biobase), autoclave (Sturdy®), oven, incubator (Incubator Hotcold- M), refrigerators, and calipers.

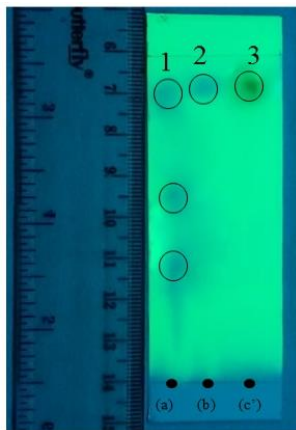


Figure 1. The elution identification of the flavonoid group at 254 nm UV light with a mobile phase of Butanol: Acetic Acid: Water (4: 1: 5). (a) Betung Bamboo Shoots Ethanol Extract (b) Betung Bamboo Shoots Sap (c) quercetin standard

The materials used in this study included bamboo shoots (*Dendrocalamus asper*), 96% alcohol, flannelette, aquadest, filter paper, silica gel 60 F254 (Merck), quercetin standards, quinine standards, butanol, acetic acid, disc paper (Oxioid), aluminum foil, MHA agar media, 0.5 Mac Farland standard, 0.9%

sterile NaCl, Amoxicillin 25 $\mu$ g / disk antibiotic (Oxioid), *Klebsiella pneumoniae* bacteria ATCC 13883, and *Pseudomonas aeruginosa* ATCC 27853.

Identification of ethanol extract compounds and bambu betung saps was done with TLC (Thin Layer Chromatography). The TLC method was used in this study because it has greater flexibility in selecting mobile phases (8). The stationary phase used was a 60 F254 silica gel plate. This plate is used because it can glow at 254 nm UV light to help visualize spots or stains on the TLC plate (9). While the mobile phase used is a mixture of Butanol: Acetic Acid: Water (4: 1: 5) (10) as shown in Figure 1.

The standard compound used to identify the flavonoid group is quercetin. Quercetin is a class of flavonoids that can be used as antibacterial because quercetin can bind to bacterial DNA gyrase, which plays a role in DNA replication. Quercetin interferes with the gyrase enzyme so that the DNA replication process stops (11). In this study, 6 series of different solution concentrations were made, both ethanol and sap extracts, namely 10% w / v, 20% w / v, 30% w / v, 40% w / v, 50% w / v, and 60% w / v.

### Results and Discussion

Characteristics of the extraction results obtained in this study were examined in organoleptic, yield calculations, and water content. The yield determination aims to quantify how much plant extract was obtained (12). A total of 150 grams of betung bamboo shoots macerated with 96% ethanol yielded 7 grams of the thick extract or around 4.67%. The extract obtained was relatively small because the main component of bamboo shoots was water. This is supported by research from Nofriyati and Ratima (6) that every 100 grams of fresh bamboo shoots contained 91% water. The characteristics of the ethanol extract of the Betung bamboo shoots can be seen in Table 1.

Table 1. Characteristics of tunas bambu betung ethanol extract

Examination		Result
Organoleptic	Consistency	Thick
	Color	Brown-yellow
	Aroma	Distinctive
Extract yield		4,67%
Water content		28,7 %

The water content requirement for an extract, according to Depkes RI (13), is less than 10%. The water content in the extract that was less than 10%; which aimed to avoid the rapid growth of the fungus in the extract (14). Based on the test results, it was found that the water content of the betung bamboo shoots ethanol extract was 28.7%, so that the resulting extract was not used for storage for a long time.

#### Identification of ethanol extract compounds and bambu betung saps

This identification test was conducted to qualitatively determine the content of secondary metabolites in the ethanol extract and sap of Betung bamboo shoots. According to Kalita et al (15), bamboo can have antibacterial activity because it has compounds of flavonoids, alkaloids, and saponins. From the results in Table 2, it can be seen that the ethanol extract and the sap of Betung bamboo shoots have quercetin compounds because the Rf value is the same (0.88). This result confirmed the research of Susilowati et al (10) that the Rf value of quercetin compounds with the mobile phase of Butanol: Acetic Acid: Water (4: 1: 5) is 0.88. Hence, it is confirmed that the ethanol extract and sap of Betung bamboo shoots positively have quercetin compounds.

Table 2. Results of the calculation of the rf value for the flavonoid group

Sample	Rf value
Tunas Bambu Betung Ethanol Extract	0.88
Betung Bamboo Shoots Sap	0.88
Quercetin standar	0.88

The identification of alkaloids is shown in Figure 2, the standard compound used was quinine. Quinine can be used as an antibacterial agent because it can interfere with peptidoglycan components in bacterial cells so that the cell wall layer is not formed entirely and causes cell death (16). The ethanol extract of betung bamboo shoots contained quinine compound because the Rf value was the same as the standard Rf value, which was 0.63 (Table 3). This confirmed the results of Susilawati et al. (10) that the Rf value of quinine compounds with the mobile phase of Butanol: Acetic Acid: Water (4: 1: 5) was 0.61. In contrast, the sap of bamboo shoots did not

have quinine compounds because its Rf value was not the same as the Rf value of quinine.

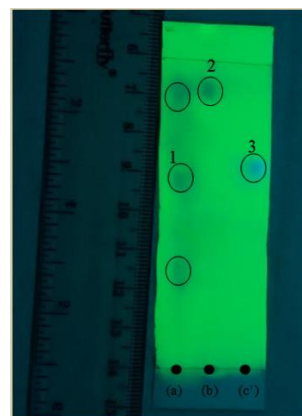


Figure 2. The results of the identification of alkaloids in UV light 254 nm with mobile phase Butanol: Acetic Acid: Water (4: 1: 5). (a) Betung Bamboo Shoots Ethanol Extract (b) Betung Bamboo Shoots Sap (c) quinine standard

Table 3. Results of the calculation of the rf value for the alkanoid group

Sample	Rf value
Betung Bamboo Shoots Ethanol Extract	0.60
Betung Bamboo Shoots Sap	0.88
Quinin standar	0.63

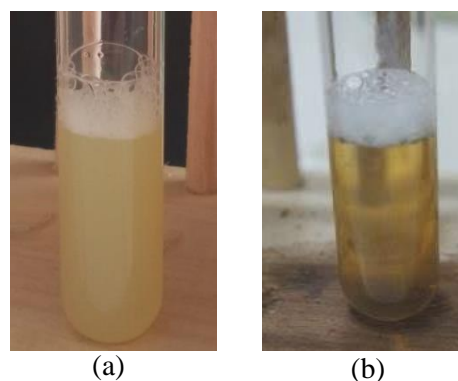


Figure 3. The results of identification of saponin compounds in the extract and sap of bamboo shoots of Betung bamboo. (a) Betung Bamboo Shoots Ethanol Extract (b) Betung Bamboo Shoots Sap

Saponin identification test was carried out by shuffling. Saponin compounds can be detected because of their ability to form foam. Saponins can damage bacterial cytoplasmic membrane cells by increasing the permeability of bacterial cell membranes (17). Based on the study results shown in Figure 3, there was 1

cm foam after being shaken for 15 minutes. Hence, it can be seen that the ethanol extract and sap of Betung bamboo shoots were positive for saponins.

#### *Antibacterial activity test of ethanol extract and saps of betung bamboo shoots*

In this study, the antibacterial activity test used the Kirby Bauer or Disc Diffusion method. This method was used because it does not require special equipment. Its implementation was more accessible and more practical, suitable for liquid samples because of the saturation of the sample on disc paper (18).

The positive control used was the amoxicillin antibiotic because amoxicillin is a broad-spectrum antibiotic that works by preventing the synthesis of bacterial cell walls (19). The agar medium used was Mueller Hilton Agar (MHA) because this media was not a selective medium so that all types of bacteria can grow (20).

When viewed from the inhibition zone category according to Davis et al (20), the results of the inhibition zone diameter

measurements of the ethanol extract of bamboo shoots against *Kliebsiella pneumoniae* were in the moderate category; while those in *Pseudomonas aeruginosa* were in the weak category. Betung bamboo shoot sap has weak inhibitory power against *Kliebsiella pneumoniae* and *Pseudomonas aeruginosa*.

Based on the table 4, the largest diameter of the inhibition zone was found in a 60% concentration solution sample, both in the ethanol extract and sap of the bamboo shoots of Betung. In the 60% ethanol extract, the inhibition zone diameter was  $9.05 \pm 0.12$  mm in *Kliebsiella pneumoniae* and  $5.07 \pm 0.13$  mm in *Pseudomonas aeruginosa*. While the sap of Betung bamboo shoots with a concentration of 60% resulted in an inhibition zone diameter of  $5.65 \pm 0.05$  mm in *Kliebsiella pneumoniae* and  $4.81 \pm 0.22$  mm in *Pseudomonas aeruginosa*. Positive control resulted in an inhibition zone diameter of  $6.09 \pm 0.03$  mm for *Kliebsiella pneumoniae* and  $5.65 \pm 0.05$  mm for *Pseudomonas aeruginosa*. The negative control used, namely sterile aquadest, did not show antibacterial activity.

Table 4. Result of inhibition zone diameter measurement

Sample	Concentration (%) b/v	Average Inhibition Zone Diameter * (mm) $\pm$ SD	
		<i>Kliebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>
Betung Bamboo Shoots Ethanol Extract	10	5.43 $\pm$ 0.40	-
	20	6.36 $\pm$ 0.39	3.16 $\pm$ 0.15
	30	6.74 $\pm$ 0.18	3.63 $\pm$ 0.46
	40	7.13 $\pm$ 0.19	3.46 $\pm$ 0.4
	50	8.10 $\pm$ 0.13	4.21 $\pm$ 0.21
	60	9.05 $\pm$ 0.12	5.07 $\pm$ 0.13
Betung Bamboo Shoots Sap	10	-	-
	20	-	-
	30	-	2.14 $\pm$ 0.07
	40	3.51 $\pm$ 0.32	2.68 $\pm$ 0.31
	50	4.53 $\pm$ 0.26	3.65 $\pm$ 0.20
	60	5.65 $\pm$ 0.05	4.81 $\pm$ 0.22
Positive control (Amoxicillin 25 $\mu$ l/disk)	-	6.09 $\pm$ 0.03	5.65 $\pm$ 0.05
Negative control (Aquadest 25 $\mu$ l/disk)	-	0	0

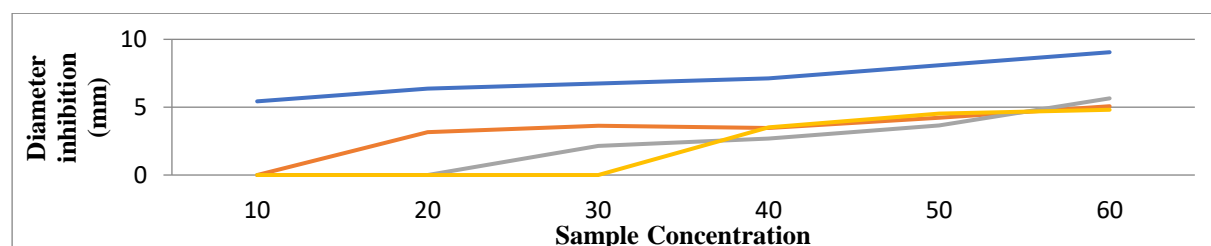


Figure 4. The graph between inhibition zone diameter and solution concentration. *Kliebsiella pneumoniae*; *Pseudomonas aeruginosa*

The diameter of the inhibition zone in the sap of the Betung bamboo shoots began to appear at a concentration of 30%, and the resulting average inhibition value was smaller than that of the ethanol extract. This can occur because the water content in the sap was higher than in the extract. The water content in the sample can affect the inhibition of bacteria because water is a medium for bacterial growth.

The greater the concentration, the greater the resulting inhibitory power because the more significant the concentration, the greater the active substance contained (21). Based on the Figure 4, it can be seen that the diameter of the inhibition zone is directly proportional to the total concentration of the solution. However, there may be a decrease in the area of the inhibition zone at a greater concentration. Research conducted by Ningtyas (21) stated that the diameter of the inhibition zone is not always directly proportional to the increase in antibacterial concentrations. This can occur due to differences in diffusion rates of antibacterial compounds on agar media and differences in the concentration of antibacterial compounds.

### Conclusion

Qualitatively, the ethanol extract and sap of the bamboo shoots (*Dendrocalamus asper*) contain flavonoids, alkaloids, and saponins. The ethanol extract of bamboo betung has a moderate bacterial inhibitory power in *Klebsiella pneumoniae* and a weak *Pseudomonas aeruginosa*. Meanwhile, the sap of bamboo shoots has weak inhibition in the *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* bacteria. It is necessary to research the activity test of the extract and sap of the shoots of bamboo betung against other bacteria, using other methods, and looking for other active compounds in the bamboo betung plant.

### Conflict of Interest

All authors declare no conflicts of interest in this section.

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