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Antibacterial Activity and Phytochemical Screening of Methanol Extract of Rambai (*Baccaurea motleyana* Muell. Arg.) Leaves from the Tropical Rainforest of East Kalimantan

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Abstract

Antibiotic resistance is a problem in the health field. Potential sources of raw materials are needed to overcome this problem. Rambai (*Baccaurea motleyana* Muell. Arg.) is one of the Baccaurea genus which is widespread throughout Asia. Several species of Baccaurea are known for their antimicrobial properties. Extraction was performed using the maceration method with methanol as solvent. Phytochemical screening of the extract was analyzed using a standard method. The antibacterial activity was tested using the agar diffusion method (Kirby-Bauer) with extract concentrations of 2, 4, 6, 8, and 10 mg/disc. The test bacteria used were *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The results showed that the extract contained alkaloids, phenolics, and flavonoids. *B. motleyana* methanol extract showed antibacterial activity at a concentration of 10 mg/disc, with an inhibition zone of 11 mm. Based on these results, *B. motleyana* leaves have potential as an antibacterial agent.

Keywords: rambai, *Baccaurea motleyana*, antibacterial, phytochemical

INTRODUCTION

Antibacterial has been known as an antibiotic, a compound used in treatment of infections caused by microorganisms (bacteria) (Mathew, 2014). Irrational uses of antibiotics are serious problem that occurs today; as a result many bacteria experience resistance (Bbosa *et al*., 2014). About 80% of society from developing countries uses traditional medicine from plants (Nascimento *et al*., 2000). The use of plants as medicine has been widely developed as an alternative therapy and a potential source of pharmaceutical ingredients (Bbosa *et al*., 2014; Sisillia, 2009). Plants provide a variety of compounds can be used as medicines (Sukemi *et al*., 2015).

Several genera of indogenus fruit plants in Indonesia, especially in East Kalimantan, have the potential as pharmaceutical raw materials such as Nephelium (Sukemi *et al*., 2015), Athocarpus (Hari *et al*. 2014), Dimocarpus (Gunawan *et al*., 2016) and Baccaurea (Rangkadilok *et al*., 2012). Species of the genus Baccaurea have been studied empirically chemical compounds. *B. motleyana* is a species of Baccaurea genera that is widespread

in eastern Kalimantan. Its fruit peels been used to treat skin diseases, anti-inflammatory and used to reduce blood sugar (Rangkadilok *et al*., 2012). Bark of *B. motleyana* is used to treat eye inflammation and antibacterial properties by local people (Sisillia, 2009). Nevertheless, research on the antibacterial activity of *B. motleyana* leaves has never been done so to find out the antibacterial activity of *B. motleyana*, this research needs to be done.

MATERIALS AND METHODS

Materials

All chemicals used are for analysis grade, Methanol (Merck®), Nutrient Agar (Merck®), NaCl (Merck®), $FeCl_3$ (Merck®), Dragendroff (Merck®), Mayer (Merck®), Mg powder (Merck®), HCl (Merck®), H_2SO_4 (Merck®), CH_3COOH (Merck®) and aquadest. Bacteria used were *Bacillus subtilis* ATTC 6633, *Escherichia coli* ATTC 25922, *Pseudomonas aeruginosa* and *Staphylococcus aureus* ATTC 25923. Fresh rambai leaves were collected in Samarinda, East Kalimantan, Indonesia. The leaves were washed gently with tap water to remove the adhering dust and soil particles. The cleaned leaves were chopped into small pieces and dried and dried using an oven with a temperature of 60ºC. A total of 200 grams of dried sample were macerated with methanol using bath technique and then filtered using filter paper, followed by evaporation using rotavapor to get a crude methanol extract.

Phytochemical Screening

Phytochemical using standard method (Harborne, 1987) alkaloid detection (Dragendraff & Mayer test); polyphenol : flavonoid (Shinoda test), phenol ($FeCl_3$ 1%); steroid/terpeneoid (Salkowski test); saponin (Frothing test).

Antibacterial Test

Antibacterial testing was carried out by diffusion method (*Kirby Bauer*) with few modification (Balouiri *et al*., 2016; Widayat *et al*., 2016). Bacterial suspensions was made following the Mc Farland 0.5 standard and concentration of the methanol extract of *B. motleyana* leaves was made using method described by Hermanda *et al*. (2016). A volume of 100 µL of bacterial suspension was homogenized with 10 mL of nutrient agar (NA) and left to produce solid media. Disc paper that concentrations of 2, 4, 6, 8, 10 (mg/disc) of extract were placed on the media and methanol was a negative control. They were incubated at 37ºC for 24 h on the incubator. Measurement of the Inhibition zone diameter at using Vernier Caliper Micrometer with three repetitions.

Statistical Analysis

The data were statistically analyzed using ANOVA followed by Tukey's HSD test ($p < 0.05$), with SPSS version 20.

RESULTS AND DISCUSSION

The methanol extraction of *B. motleyana* leaves yielded 31.25 g of crude extract (Table 1). The obtained extract exhibited a viscous, green-colored appearance with a calculated extraction yield of 15.63% (w/w). This substantial yield suggests good extractability of bioactive compounds using methanol as the solvent.

Table 1. Yield value rambai leaves (*B. motleyana*) extract

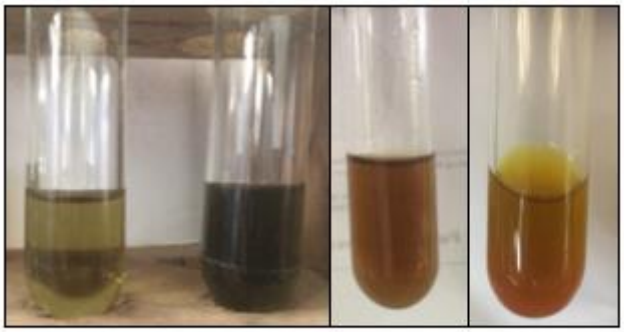
Sampel Name	Sampel Weights (gram)	Annotation
Dried Rambai Leaves	200	Powder
Rambai Leaves	31.25	Extract

Qualitative analysis of the methanol extract from *B. motleyana* leaves confirmed the presence of alkaloids, phenolics, and flavonoids (Table 2; Figure 1), while saponins and steroids/terpenoids were undetected, potentially due to concentrations below the assay's detection limit or inefficient extraction of these specific compound classes.

Table 2. Phytochemicals screening results of rambai leaves metahnol extract (*B. motleyana*)

Secondary Of Metabolite					
Alkaloid		Steroid/ Terpenoid	Polyphenols		Saponins
Mayer	Dragendorf		Flavonoid	Phenolic	
-	+	-	+	+	-

(+) : Detected
(-) : Not Detected



A B C D

Figure 1. Phytochemicals screening results (A) extract; (B) phenolic; (C) flavonoid; (D) alkaloid

The results demonstrated that the methanol extract of *B. motleyana* leaves exhibited significant antibacterial activity against two Gram-positive and two Gram-negative bacterial strains (Figure 2). According to established criteria (Dall'Agnol *et al.*, 2003;

Rostinawati *et al*., 2018), plant extracts are classified as having strong antibacterial potential if their minimum inhibitory concentration (MIC) is below 100 mg/mL, moderate activity at 100–500 mg/mL, and weak effects at 500–1000 mg/mL, while concentrations exceeding 1000 mg/mL are considered ineffective. Notably, the observed efficacy of *B. motleyana* extract aligns with these benchmarks, suggesting its promising role as a natural antimicrobial age (Rostinawati *et al*., 2018).

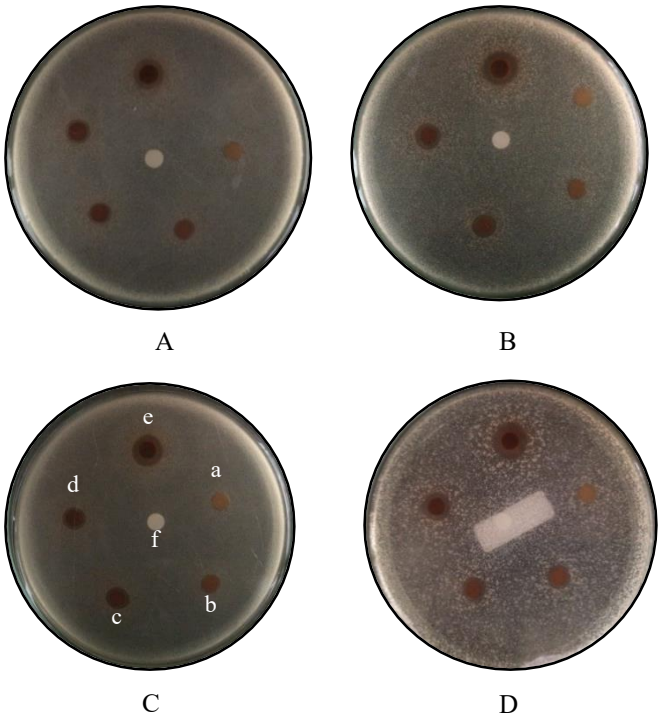


Figure 2. Results of rambai leaves (*B. motleyana* Muell. Arg) methanol extract test against bacteria: (A) *B. subtilis*, (B) *E. coli,* (C) *P. aeruginosa*, (D*) S. aureus*, (a) 2 mg/disc; (b) 4 mg/disc; (c) 6 mg/disc; (d) 8 mg/disc; (e) 10 mg/disc; (f) Negative control

Based on the inhibition zone diameter, the methanol extract of *B. motleyana* leaves exhibited detectable antibacterial activity starting at a concentration of 4 mg/disc, with an average zone diameter of 6–7 mm. The highest inhibitory effect was observed at 10 mg/disc, producing a more pronounced zone of 10–11 mm, whereas the lowest tested concentration (2 mg/disc) showed no activity. These findings suggest a concentration-dependent antibacterial response, where efficacy escalates with increasing extract dosage.

The antibacterial activity of the *B. motleyana* leaf extract at each tested concentration yielded statistically significant results (Table 3). At 10 mg/disc, the inhibition zone diameters varied across bacterial strains, ranked from highest to lowest as follows: *S. aureus* > *E. coli* > *P. aeruginosa* > *B. subtilis*. This hierarchy of sensitivity suggests that the extract possesses broad-spectrum antibacterial activity, with notable efficacy against

both Gram-positive (S. *aureus, B. subtilis*) and Gram-negative *(E. coli*, *P. aeruginosa*) pathogens.

Table 3. Mean diameters of inhibition zones for each concentration of methanol extract from *Baccaurea motleyana* leaves

Bacteria	Diameter of Inhibition Zone (mm)				
	2 mg/disc	**4 mg/disc**	**6 mg/disc**	**8 mg/disc**	**10 mg/disc**
B. subtilis	0±0.00^{c}	7.180±0.19^{b}	7.627±0.19^{b}	8.252±0.08^{a}	9.343±0.13^{a}
E. coli	0±0.00^{c}	6.840±0.23^{b}	7.942±0.47^{b}	8.445±0.33^{a}	10.869±0.30^{a}
P. aeruginosa	0±0.00^{c}	7.151±0.14^{b}	7.887±0.56^{b}	8.555±0.29^{a}	10.686±0.99^{a}
S. aureus	0±0.00^{c}	7.335±0.35^{b}	8.067±0.43^{b}	9.257±0.61^{a}	11.996±0.11^{a}

**Analysis of variance (ANOVA), Tukey's HSD test ($p < 0.05$); means with the same superscript letter within a row are not significantly different.*

The antibacterial activity of *B. motleyana* leaves stems from its rich phytochemical composition, with different compound classes acting through distinct mechanisms. Alkaloids exert their antimicrobial effects by intercalating into bacterial cell walls and DNA, thereby inhibiting replication processes and disrupting proper cell wall formation, ultimately leading to cell death (Cohen, 1992; Compean and Ynalvez, 2014; Cushnie & Lamp, 2005; Dall'Agnol *et al*., 2003; Davidson & Branen, 1993; Gunawan *et al*., 2016; Bbosa *et al*., 2014; Harborne, 1987). Phenolic compounds demonstrate antibacterial activity by damaging microbial cell walls and membranes, effectively inhibiting bacterial growth through structural compromise (Scalbert, 1991) As a significant subgroup of phenolics, flavonoids contribute to antibacterial action through multiple pathways including protein denaturation that halts metabolic activity, and cytoplasmic membrane damage that inactivates essential bacterial enzyme systems (Cushnie & Lamb, 2005). Notably, flavonoids represent nature's most abundant and diverse group of antibacterial compounds, playing a crucial role in plant defense mechanisms (Wafa *et al*., 2016; Rostinawati *et al*. 2018). The combined action of these phytochemicals results in broad-spectrum antimicrobial activity against various pathogenic bacteria.

The results indicate that *B. motleyana* leaves possess significant antibacterial potential. As an initial screening study, this research has identified promising antimicrobial activity in the leaf extracts. Current work involves fractionation using solvents of increasing polarity to determine the minimum inhibitory concentration (MIC) values. The most active fraction will then undergo further characterization through TLC-bioautography to localize active compounds, followed by structural identification of the bioactive constituents.

CONCLUSION

This study demonstrates that the methanol extract of B. motleyana leaves exhibits significant antibacterial activity against both Gram-positive (*B. subtilis* and *S. aureus*) and Gram-negative (*E. coli* and *P. aeruginosa*) bacterial strains. At concentrations of 4-10 mg/disc, the extract effectively inhibited bacterial growth, with the highest efficacy observed at 10 mg/disc. These findings suggest that *B. motleyana* leaves contain bioactive compounds with potential for development as natural antibacterial agents..

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CONFLICTS OF INTEREST

The authors declare no conflict of interest, research conducted following the provisions of the funder.

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