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Microbial resistant of building plants of *Gigantochloa apus*

Retno Indriatie[1,2], Siti Mudaliana[1] and Masruri[2*]

[1]UPT Herbal Laboratory of Materia Medica, Batu, Indonesia.
[2]Chemistry Department, Brawijaya University, Jl. Veteran 65145 Malang, Indonesia

*Corresponding Author: masruri@ub.ac.id

Abstract. The plant of *Gigantochloa apus,* has been traditionally used as a material for building houses, floor, roof top, fence and bamboo-crafts. In practice, beside the strength and elasticity, stability towards microbial also becomes an important consideration. This paper reports the chemical composition of the *Gigantochloa apus* and its resistance to bacterial growth. It was found that *Gigantochloa apus* contained alkaloids and phenolic tannins which are predicted to deter microbes. The ability to resist bacterial growth was evaluated in two common model microbes; i.e. *Escherichia coli* and *Staphylococcus aureus*. The chemicals afforded from *Gigantochloa apus* have capability to inhibit bacterial growth eight times stronger than the reference compound.

1. Introduction

Plant species *of Gigantochloa apus* have been widely used by locals for building houses [1, 2] over several decades such as for particle board materials [3, 4], and furniture [5]. Recently, many handcraft products [4–6] were also developed and marketed by using *Gigantochloa apus* as the main material (Fig 1). *Gigantochloa apus* material composed of cellulose which is responsible for the strength and elasticity of its products [1, 2, 7, 8]. However, other chemicals also exist in the *Gigantochloa apus* which are known to be responsible for its stability and durability [9–11] towards microbial decomposition [12]. Many reports have indicated that secondary metabolites contained in the *Gigantochloa apus* are responsible for this property [13, 14]. So far, many strategies have been developed to improve this activity [15–17]. Other species of *Gigantochloa apus* have been reported for their activity in different extraction solvents and conditions [14]. Phenolics [14], saponins, tannins and alkaloids from secondary metabolite groups [11, 12, 18] were detected in the extract. This paper reveals chemicals composition in the *Gigantochloa apus* that are responsible for the capability to inhibit decomposition by microbial.

2. Experimental procedure

2.1. Extract preparation

The *Gigantochloa apus* is determined as *Gigantochloa apus* (Schult.) Kurz in UPT Herbal Laboratory of Materia Medica Batu, Indonesia. The sample was bought from a local market in Batu as a young shoot. It was further dried in oven and then powdered. The extraction was done using a fast pressurized solvent extraction (Buchi, Switzerland) in ethanol. A 5 g of sample was extracted with a 25 mL of ethanol 96%. Preheating at 60 °C was conducted and repeated in three cycles to afford the ethanol solution of the extract. It was further concentrated under reduced pressure using a rotary evaporator (Buchi, Switzerland). The dried extract was obtained as a brown-semi solid extract. Several

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concentrations were also prepared from 50 mg/mL of stock solution of the dried extract into 1.25 mg/mL, 2.5 mg/mL and 5.0 mg/mL.



Figure 1. Materials characteristic of *Gigantochloa apus* for handicrafts.

2.2. Chemical analysis

The procedure to analyze the chemicals composed of the extract of *Gigantochloa apus* was derived from a reference reported by [19].

2.2.1. Flavonoid analysis. The dried extract was dissolved in distilled water and a 1.0 mL of sample was heated for 5 minutes. A 10 mg of magnesium was added followed by dropwise addition of 38% hydrochloric acid solution. The positive flavonoid was detected due to change of color in the solution from orange to red.

2.2.2. Alkaloid analysis. The dried extract was dissolved in distilled water. A 1.0 mL of sample in a reaction tube was added with Meyer reagent. The different tubes were also prepared and added with Dragendorf reagent and Bouchard reagent. The formation of precipitate indicated alkaloid presence in the sample.

2.2.3. Saponin analysis. The dried extract was dissolved in distilled water. A 1.0 mL of sample in reaction tube was added with 1.0 mL of hot water and 3 drops of 35% hydrochloric acid solution. The formation of stable foam indicated the presence of triterpenoid saponin.

2.2.4. Tannin analysis. The dried extract was dissolved in distilled water. A 1.0 mL of sample was heated for 5 minutes and a 1.0% of iron(III) chloride solution was added. The change of color to greenish brown or blackish blue indicated the presence of tannin.

2.3. Microbial-growth inhibition evaluation

Growth inhibition of the extract was analyzed following the Kirby-Bauer disk diffusion susceptibility test [20]. Bacteria species of *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as model. The paper disk was immersed in the extract sample (concentration 1.25 mg/mL; 2.5 mg/mLand 5.0 mg/mL), gentamicin 40 mg/mL, and sterilized water. Then, the disks were placed into inoculated bacteria. Further incubation was conducted at 37 ^{o}C for 2 x 24 hours. The growth inhibition activity was determined from the clear zones around the paper disk.

3. Results and discussion

3.1. Chemical analysis

Cellulose and lignin are found as the main chemicals of *Gigantchloa apus*. Both chemicals were identified from the fresh and dried samples (Table 1). They are also used as the main backbone for preparing handcraft product materials. The other organic extract (4.64-4.88%) was isolated from ethanol/toluene as solvent [21].

Table 1. Chemical composition of *Gigantochloa apus.*

Sample condition	**Cellulose (%)** [21]	**Lignin (%)** [21]	**Organic extract (%)** [21]
Dried	72.65	22.71	4.64
Fresh	69.73	25.39	4.88
Secondary metabolite	**Leaf** [22]	**Dried** [12]	**This study**
Flavonoid	-	-	-
Alkaloid	+	-	+
Saponin	+	-	-
Tannin	-	+	+
Terpenoid	+	-	-

Identification of the composition of the organic extract was previously reported to contain alkaloid and saponin in the leaf [22]. Meanwhile, tannin is detected from the dried sample [12]. Our investigation found alkaloid and tannin as the main chemical composition of the organic extract (Fig. 2). These compounds are predicted to be responsible for stabilizing the materials of *Gigantochloa apus* against destruction by microbes.

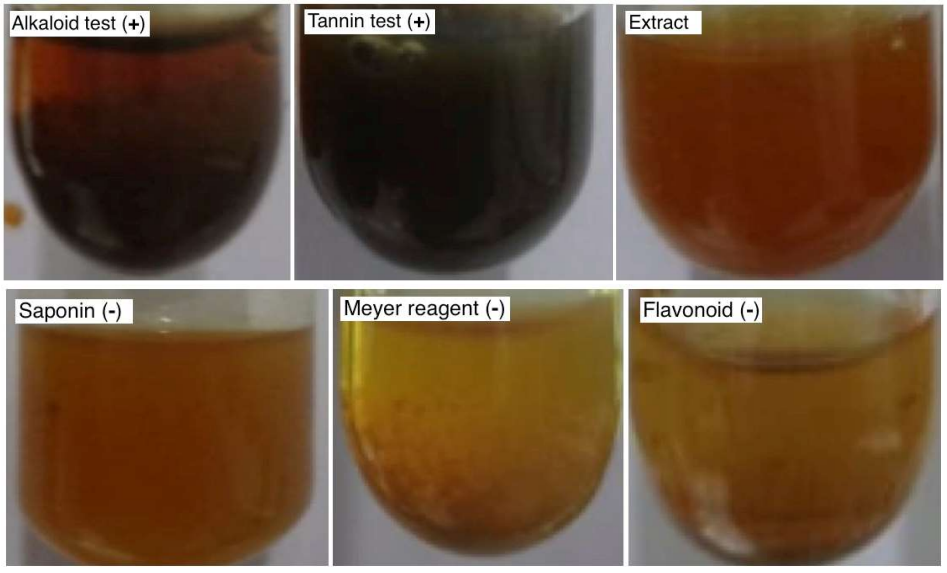

Figure 2. Visualisation of the chemicals compose in *Gigantochloa apus*. Alkaloid and tannin positive contains in the sample.

3.2. Bacterial growth inhibition evaluation

The Kirby-Bauer method is applied to analyze activity of chemicals composed in the *Gigantochloa apus*. In all concentrations, the extract is active for decrease in bacterial development. The activity is higher than gentamicin in both bacterial models, *Escherichia coli* and *Staphylococcus aureus* (Table 2).

Table 2. Growth inhibition activity of chemicals from *Gigantochloa apus.*

Evaluated sample	Bacterial growth inhibition capability (mm)	
	E. coli	***S. aureus***
Control (water)	0	0
G. apus extract (1.25 mg/mL	1.71	7.72
G. apus extract (2.50 mg/mL)	2.45	8.60
G. apus extract (5.00 mg/mL)	3.94	10.52
Gentamicin (40.0 mg/mL)	2.58	6.00

The visual appearance for bacterial growth inhibition is shown in Fig 3. The extract concentration of 5.0 mg/mL shows a wider and clear zone around the paper disk. The bacteria are inhibited from growing in this zone.

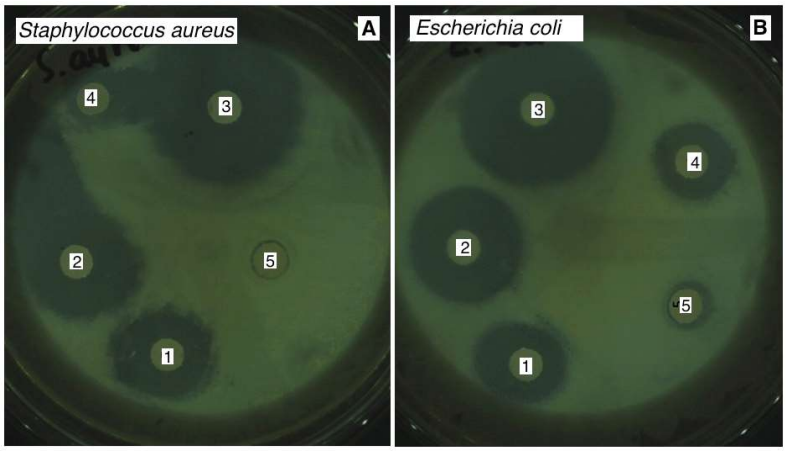

Figure 3. Photograph growth inhibition of *Gigantochloa apus* extract in *Staphylococcus aureus* (A) and *Escherichia coli* (B). Control only contain water (5) and sample in variation concentration (1-3), and gentamicin (4).

4. Conclusion

The *Gigantochloa apus* contains cellulose and lignin as main chemicals with the minor organic extract composed of alkaloids and tannins. These compounds belong to the class of secondary metabolites. They are predicted to be responsible for the stability and ability to inhibit the deterioration of *Gigantochloa apus*-based materials for handicraft.

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