Antibacterial Activity of Methanol Extract of *Calotropis gigantea* Flowers from Aceh

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Abstract: The purpose of this study was to determine the antibacterial activity of methanol extract of *Calotropis gigantea* flowers at various concentrations of gram-positive bacteria (*Staphylocccus aureus*) and gram-negative bacteria (*Escherichia coli*). This study used a completely randomized design (CRD) consisting of 5 treatment groups and 2 control groups with 4 repetitions. The treatment group consisted of *Calotropis gigantea* methanol extract with a concentration of 10%, 25%, 50%, 75% and 100% and the control group consisted of negative control using 1% Sodium Carboxy Methyl Cellulose (CMC) and positive control using ampicillin for *S. aureus* and chloramphenicol for *E. coli* bacteria. Antibacterial activity test was carried out by disc diffusion method. Phytochemical test results showed that the methanol extract of *Calotropis gigantea* flower contained alkaloids, steroids, terpenoids, flavonoids, saponins, tannins and coumarin. Antibacterial test results of methanol extract of *Calotropis gigantea* flower on *Staphylocccus aureus* and *Escherichia coli* showed that the methanol extract of *Calotropis gigantea* flowers had activity against *Staphylocccus aureus* and *Escherichia coli* bacteria and increased concentration of methanol extract of *Calotropis gigantea* flower followed by increased inhibition zone. Statistical analysis with ANOVA and Duncan test at 95% Confidence Interval showed significant differences between treatment groups.

1 INTRODUCTION

Indonesia is a country rich in biodiversity, one of which is vegetation. The role of plants has provided many benefits for humans in the field of medicine, one of which is the *Calotropis gigantea* plant (*C. gigantea*). This plant is a shrub from the family Apocynaceae which is often found in the regions of Bangladesh, Burma, China, India, Indonesia, Malaysia, Pakistan, Philippines, Thailand and Sri Lanka (Sarkar et al., 2014). Some parts of this plant such as leaves, stems, roots and flowers are traditionally used to treat various diseases such as tumors, fever, rheumatism, digestive disorders, coughs, flu, asthma, nausea and diarrhea (Patel et al., 2004). Ethnobotany part of the root of this plant is efficacious to increase the flow of bile to the intestine, healing skin infections, intestinal worms, coughing asthma, bronchitis and dyspepsia (Kumar et al., 2011).

A report stated that in India these plant roots are used for sedatives, anticonvulsants, fever, laxatives and deworming drugs (Argal et al., 2006). Plant roots of *C. procera* which is a genus with the plant *C. gigantea*, can treat colds and coughs, syphilis and elephantiasis, can also function as anti-inflammatory, analgesic, antimalarial and antimicrobial (Kumar, 2009). Generally plants that are in one genus such as *C. gigantea* and *C. procera* contain the same secondary metabolites (Wink, 2010). Methanol extract of biduri plant roots caused death in *Aedes aegypti* larvae (Zanaria et al., 2012). Ethanolic extract of *C. gigantea* plant roots can heal wounds faster in mice and this is in accordance with the traditional use of *C. gigantea* root plants in India, namely for wound healing (Deshmukh et al., 2009). The methanol extract of this plant was active as an insecticide against the larvae of *Tribolium castaneum* (red flour beetle) (Alam et al., 2009). *C. gigantea* flower extract has the potential as an antimicrobial agent in the presence of terpenes, where the activity of the extract was seen from the Muller Hinton agar medium inhibition zone against *Pseudomonas aeruginosa*, *E. coli*, and *Tinea capitis* (Patil, 2012). Based on phytochemical analysis of

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bioactive compounds *in vitro* in *C. gigantea* acts as an antibacterial against many bacteria such as *B. cereus*, *B. subtilis*, *E. coli*, *K. pneumonia*, *S. aureus*, *S typhii*, *Micrococcus luteus* in all alkaloid extracts, cardiac glycosides, tannins, saponins, flavonoids, steroids, terpenoids sugar and resins (Seniya et al., 2011). *C. gigantea* latex extract shown very good fungicidal effects thus indicating that latex can be a useful source for the development of new antifungal agents against pathogenic fungi (Saratha & Subramanian, 2010). Thus, the researcher were interested in conducting an antibacterial activity test from the methanol extract of *Calotropis gigantea* flowers originating from Aceh against gram-positive bacteria *Staphylocccus aureus* and gram-negative bacteria *Escherichia coli*.

2 METHODS

2.1 Collection of *C. gigantea* Flowers

Calotropis gigantea flowers was collected from Kayee Jatoe Cubo village dengan ketinggian 150 m diatas permukaan laut, Kecamatan Bandar Baru, Kabupaten Pidie Jaya during October 2017. The plant was identified in the Herbarium Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Syiah Kuala University (Reg. No of the certificate: No. 943/UN11.1.28.1/DT/2017). The collected plant parts (flowers) were separated from undesirable materials. The flowers were dried in open air under shade for one week.

2.2 Tools and Materials

The tools used for this study were autoclave, incubator, refrigerator, analytic scale, blender, vacuum rotary evaporator (Heidolph), vortex, spectrophotometer, ruler, petri dishes, test plates, glass ware, micropipets, swab, scissor, label paper and gloves.

The materials used in this study were methanol, *Calotropis gigantea* flowers, gram positive bacteria (*Staphylocccus aureus*) and gram negative bacteria (*Escherichia coli*) obtained from the Aceh Provincial Health Laboratory, blank discs, ampicillin and chloramphenicol discs, 96% alcohol, aluminum foil, Wagner, Mayer, and Dragendorf reagent, magnesium powder, chloroform, sulfuric acid, Lieberman-Burchard reagent, sterile aquadest, Nutrient Agar (NA), Muller Hinton Agar (MHA) media, sterile stick cotton.

2.3 Extraction Procedure

Calotropis gigantea flowers which have been dried as much as 2 kg are smoothed into a blender until they become powder then macerated with methanol for 3x24 hours. The maserate obtained was concentrated with a vacuum rotary evaporator at a temperature of 50-60 ^{0}C until a concentrated extract was obtained. *Calotropis gigantea* flowers extract 100% pure diluted in various concentrations, namely: 10, 25, 50, 75 and 100%.

2.4 Phytochemical Analysis

The fresh flowers and concentrated methanol extracts have been conducted a phytochemical test as Harborne phytochemical method to see whether they contain secondary metabolites (Table 1) (Harborne, 2006).

2.5 Antibacterial Activity Test

Antibacterial activity test was carried out by Kirby-Bauer disc diffusion method. This test uses Muller Hinton Agar (MHA) media with a depth of 4 mm (25 ml of MHA media for 100 mm petri dishes and 60 ml for 150 mm petri dishes) (Hood et al., 2010). The bacterial suspension that will be used for antibacterial power tests is measured in density using a spectrophotometer (λ = 625 nm and absorbance 0.08 - 0.13) to obtain the bacterial density standard at 1-2 x 10^8 CFU/ml as stated by European Committee on Antimicrobial Susceptibility Testing (Kahlmeter, 2006). Then the bacteria were inoculated into the MHA media by dipping sterile stick cotton into the inoculum and then rubbing it on the entire surface of the MHA media three times by rotating the cup at an angle of 60 ^{0}C after each application so that the flat media surface was filled with bacterial suspension. Control discs, antibiotic discs (ampicillin or chloramphenicol) and discs containing various concentrations of samples were placed on the inoculation media using sterile tweezers and pressed slowly and incubated at 37 ^{0}C for 24 hours. After incubation, the diameter of the inhibitory zone in the form of clear zone is measured using a ruler in millimeters (mm) and compared with the inhibition power classification (Morales et al., 2003).

2.6 Data Analysis

The data obtained in this study, in the form of inhibitory zone diameter growth of *Staphylocccus aureus* gram-positive bacteria and *Escherichia coli* gram-negative bacteria in various concentrations of methanol extract of *Calotropis gigantea* flowers tested for normality with Kolmogorov-Smirnov test and homogenity test with Lavene test. Then ANOVA test was conducted at 95% Confident interval (CI) and continued with Duncan test.

3 RESULTS AND DISCUSSION

3.1 Total Yield Extracts and Its Color

Extraction 2 kg flowers of *C. gigantea* produced 1.97% methanol extract with greenish yellow color.

3.2 Phytochemical Constituents

C. gigantea flowers and methanol extract of *C. gigantea* flowers contain many secondary metabolites. Phytochemical test results for *C. gigantea* fresh flowers contain alkaloids, streroids, saponins, phenols, flavonoids, tannins and coumarin. whereas the methanol extract of *C. gigantea* flowers obtained secondary metabolites of alkaloids, steroids, phenols, flavonoids, tannins and coumarin (Table 2). This shows that methanol as a polar solvent can attract many compounds which have the potential to be used as drugs.

Table 1. Phytochemical Test of Flower and Methanol Extract of *C. gigantea* Flower

Chemical content	Reagent	Phytochemical Test Results of *C. gigantea* flowers	Phytochemical Test Results of Methanol Extract *of C. gigantea* flowers	Information
Alkaloid	Mayer	+	+	White sediment
	Wagner	+	+	Chocolate sediment
	Dragendorff	+	+	Red sediment
Steroid	Liebermann-Burchard Test	+	+	Green or blue
Terpenoid	Liebermann-Burchard Test	-	-	Red
Saponin	Shuffle	+	-	Foaming
Phenol	$FeCl_3$ 5%	+	+	Deep Blue or Black
Flavonoid	Mg dan HCl	+	+	Red
Tannin	Dilute HNO_3	+	+	Change from Reddish to Yellow
Coumarin	Ammonia Steam	+	+	Presence of fluorescence

+ present - absent

The presence of tannins shows the ability of this plant to play an important role as an antidiarrheal and antihemorhagic agent (Asquith & Butler, 1986). Phenol compounds are known to have several subclasses, namely simple phenols, phenolic acids, quinones, flavonoids, flavones, flavonols and tannins (Cowan, 1999). Phenol compounds are known to have antibacterial properties. Mechanism phenol compounds as antibacterial substances are by penetrating the cell wall and into the cytoplasm and then precipitate the protein in the microbial cell. Phenol compounds can also denaturize enzymes that function in germination of spores or affect the amino acids responsible for the germination process. Large molecular phenolic compounds are able to activate essential enzymes in microbial cells even in low concentrations. Phenol compounds can break the peptidoglycan bond when breaking through the cell wall. This will cause cell lysis (Hariyati, 2010).

The antimicrobial activity of the methanol extract of *C. gigantea* flowers studied in this study was assessed qualitatively by measuring the diameter of the inhibition zone.

Table 2. Antibacterial activity test results of methanol extract of *C. gigantea*

Treatment / Control	Concentration (%)	Inhibitory Zone Diameter (mm)		Classification based on Morales et al.
		S. aureus	*E. coli*	
T_1	10	6	7	+
T_2	25	11	17	++
T_3	50	18	19	++
T_4	75	23	22	+++
T_5	100	28	25	+++
C_-	CMC 1%	0	0	-
C_+	Antibiotics	24 (Ampicillin)	30 (Chloramphenicol)	+++

Information:
T_1 : Methanol Extract of *C. gigantea* flowers with 10% concentration
T_2 : Methanol Extract of *C. gigantea* flowers with 25% concentration
T_3 : Methanol Extract of *C. gigantea* flowers with 50% concentration
T_4 : Methanol Extract of *C. gigantea* flowers with 75 % concentration
T_5 : Methanol Extract of *C. gigantea* flowers with 100% concentration
C_- : CMC 1% as negative control
C_+: Antibiotics as positive controls (ampicillin for *S. aureus*; chloramphenicol for *E. coli*)

3.3 Antibacterial Activity

The antimicrobial activity of the methanol extract of *C. gigantea* flowers studied in this study was assessed qualitatively by measuring the diameter of the inhibition zone. To determine the zone of inhibition, Gram-positive, Gram-negative were taken as standard antibiotics (ampicillin, chloramphenicol) for comparison of results. The results of this study revealed that the extracts of the plants tested had potential antimicrobial activity against Gram-positive and Gram-negative microorganisms.

4 CONCLUSIONS

Methanol extract of *C. gigantea* has antibacterial activity against *S. aureus* and *E. coli* bacteria and also increasing the concentration of methanol extract of *C. gigantea* flowers followed by an increase in inhibitory zones.

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