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Antimicrobial Activity and Toxicity Test of *Baccaurea angulata* Leaves

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Abstract: *Baccaurea angulata* is one of the underutilized plant species in Indonesia. *Baccaurea angulata* is a medicinal plant that produces active compounds and has the potential to inhibit the growth of bacteria and fungi that cause infectious diseases. Flavonoids are one of the secondary metabolites found in the leaves of *Baccaurea angulata*. These compounds are used as an anticancer, antitumor, wound infection medicine, antiviral and antifungal. This study aims to determine the antimicrobial activity and toxicity of *Baccaurea angulata* leaves originating in Ceruk Village, Northeast Bunguran District, Natuna Regency, Riau Islands. This leaf extract is extracted by maceration with methanol as a solvent. Antimicrobial test using disc paper diffusion method (Kirby-Bauer / KB) with various concentrations of 40%, 60%, 80%, and 100%. The Toxicity test using the BSLT method with *Artemia salina* Leach larvae as test animals. The results showed that *Baccaurea angulata* leaf extract concentration of 100% had an antimicrobial activity that could inhibit the growth of *Escherichia coli* ATCC 25922 with a diameter of 12.3 mm, *Staphylococcus aureus* ATCC 25923 14.4 mm, *Candida albicans* ATCC 10231 15.56 mm, and the toxicity test obtained an LC50 value of 605.29µg/ml. The conclusion is that *Baccaurea angulata* leaf extract has antimicrobial activity and is toxic to *Artemia salina* Leach shrimp larvae.
Keywords: *Baccaurea angulata*, Antimicrobial, Kirby-Bauer (KB) method, Brine Shrimp Lethality Test (BSLT)

I. INTRODUCTION

Plants are part of natural resources and have a role in human life. One of the important roles of plants in the industrial world is as a raw material for medicines. Medicinal plants are plants that are efficacious or have medicinal properties, and these properties are known based on the narrative of their parents or from experience (Fitriatien et al. 2017). One of the efficacious plants is *Baccaurea angulata* Merr, Indonesian society is known as Dayak Belimbing, Jungle Belimbing, or Ucung. This plant is one of the underutilized plants in Indonesia. (Andriyanto et al., 2016)

Infectious disease is caused by the entry of pathogenic microorganisms into the body, usually caused by fungi and bacteria. Some several bacteria and fungi can cause infection in humans, including *Escherichia coli* bacteria, which are Gram-negative bacteria that cause diarrheal disease and *Staphylococcus aureus* bacteria, which are Gram-positive bacteria that cause skin diseases such as boils, and the fungus *Candida albicans*, which causes candidiasis.

Antimicrobial is a substance that kills or inhibits microorganisms such as bacteria or mold and is usually found within an organism as a secondary metabolite. The mechanism of antimicrobial compounds is generally carried out by damaging cell walls, changing membrane permeability, disrupting protein synthesis, and inhibiting enzyme action (Pelczar and Chan, 2008). These compounds that can damage cell walls include phenols, flavonoids, and alkaloids. These phytochemical compounds have the potential as natural antimicrobials for pathogenic bacteria, such as *Escherichia coli* and *Staphylococcus aureus*. *Baccaurea angulata* leaves contain secondary metabolites, such as alkaloids, which interest researchers in conducting antimicrobial tests on *Baccaurea angulata* leaves.

Brine Shrimp Lethality Test (BSLT) is a screening method to determine the toxicity of an extract or natural compound, based on the killing ability of test compounds on simple zoological organism-brine shrimp (*Artemia salina* Leach), due to the influence of extracts or compounds of natural ingredients at a given concentration. Flavonoids are one of the secondary metabolites found in plants. This compound used as an anticancer, antitumor, wound infection medicine, antiviral and antifungal.

II. MATERIALS AND METHODS

2.1 Plant material collection, Preparation and Authentication

Baccaurea angulata leaves were collected at Ceruk Village, Northeast Bunguran District, Natuna Regency, Riau Islands Indonesia. The identification process has been carried out at the Biology Herbarium, Faculty of Mathematics and Natural Sciences, Andalas University Padang, with identification number 483/K-ID/ANDA/X/2020. *Baccaurea angulata* leaves are cleaned of dirt, then washed thoroughly using running water so that no dirt is left behind, then dry the sample in aerated way. After drying, the leaves were grinded into a fine powder. The powder was stored in an air-tight container for further use. The powdered plant material was extracted successively with methanol, and then vacuum dried in a rotary evaporator (Heidoph). All steps were carried out in dark condition.

Fig. 1: *Baccaurea angulata* plant

2.2 Phytochemical Screening

Phytochemical analysis of the extract was determined by the methods screening tests of tannins, flavonoid, alkaloids, saponin, steroid, and terpenoid as described by Harborne (1998)

Table 1. Phytochemical detection

Phytochemical test	Detection
Test for alkaloids (Dragendorfs' test)	1 g of extract was dissolved in 5 mL of HCl (1.5% v/v) and permeated. These filtrates were then used for testing alkaloids. Dragendorfs' reagent was added into 2mL of filtrate. The formation of orange-brown precipitate indicated the presence of an alkaloid.
Test for flavonoids	In a test tube with 1 g of extract, a few drops of NaOH were added and shaken. An intense yellow color was produced in the plant extract, which became colorless on adding a few drops of dilute acid, indicating the presence of flavonoids.
Test for saponins (Foam test)	1g of extract was shaken with distilled water in a were taken as preliminary evidence for the presence of test tube for 15mins. Bubbles that persist on warming saponins.
Test for steroid (Salkowaski test)	1g of extract was dissolved in 2 mL of $CHCl_3$, and 2 mL of concentrated H_2SO_4 was added from the side of the test tube. The test tube was shaken for a few minutes. The development of red color in the $CHCl_3$ layer indicated the presence of steroid.
Test for tannin	1g of extract was stirred with 10mL of distilled water, filtered, and 1mL of 5% $FeCl_3$ was added to the filtrate. A blue-black, green, or blue-green precipitate was taken as evidence of the presence of tannins.
Test for terpenoid	5g of extract was mixed with 2 mL of $CHCl_3$. 3mL of concentrated H_2SO_4 was added to form a layer. A reddish-brown precipitate coloration at the interface formed indicated the presence of terpenoids.

2.3 Microbial strain

The test organisms are *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Candida albicans* ATCC 10231.

2.4 Antimicrobial Activity Testing

Antibacterial and antifungal activity testing was carried out using the Kirby-Bauer (KB) method against *Staphylococcus aureus, Escherichia coli* for bacteria, and *Candida albicans* for fungal. The concentration extract of the following amounts 40%, 60%, 80%, and 100%. Positive control bacteria testing is Chloramphenicol 5%, and for fungal is Ketoconazole 10%, whereas DMSO 10 % is a negative control.

Bacteria culture solutions contain a turbidity standard of 0.5 x 106 CFU/ml prepared in normal saline and spread on sterile Mueller Hinton agar plates using the spread plate technique. Then discs containing different concentrates extract, Chloramphenicol, and DMSO were put on an agar plate where the selected bacteria were grown. The plates were incubated at 37°C for 24 hours. After incubation, the zone of inhibition was recorded. All the tests were in triplicates and were repeated three times.

Fungal culture solutions contain a turbidity standard of 0.5 x 106 CFU/ml prepared in normal saline and spread on sterile PDA agar plates using the spread plate technique. Then discs containing different concentrates extract, Ketoconazole, and DMSO were put on an agar plate where the selected fungal were grown. The plates were incubated at 37°C for 24 – 72 hours. After incubation, the zone of inhibition was recorded. All the tests were in triplicates and were repeated three times.

2.5 Brine Shrimp Lethality Test (BSLT)

The BSLT test concentrations were 500 ppm, 250 ppm, 125 ppm, 50 ppm, and 0 ppm (as a negative control). Preparation of a stock solution, 50 mg extract dissolved in 50 ml of seawater, until a stock solution concentration of 1000 ppm was obtained. Ten brine shrimp larvae were added to each vial, with 50 ppm of yeast suspension as food. The vials were placed under the heat lamp and left for 24 h with observation at 0, 1, 2, 3, 4, 5, 6, 12, 18, and 24 hours. Every interval of dead larvae must count using a loupe (larvae that did not show movement for 10 seconds were declared dead, and the percentage calculated of the dead larvae was. Each extract concentration was repeated three times and determined the LC50 value with probit analysis using the Statistical Package for the Social Sciences (SPSS). An extract is declared active if it has an LC50 value <1000 ppm. The larvae used are 48 hours old because, at this age, the larvae have complete limbs.

III. RESULTS AND DISCUSSION

Plants are a significant source of various health-beneficial phytochemicals such as flavonoids, phenols, saponins, alkaloids, vitamins, minerals, and carbohydrates. The plant-based medicines are common as a source of primary healthcare in many parts of the world, especially Africa, Asia, and parts of America, because they are rich in phytochemicals and secondary metabolites for medicinal properties. A thousand of phytochemicals, such as alkaloids, phenolics, flavonoids, saponins, anthocyanins, and terpenoids, have been isolated and identified from numerous plants due to pharmacological properties

3.1 Phytochemical Screening

The phytochemical screening test for *Baccaurea angulata* extract showed positive results for secondary metabolites of flavonoids, alkaloids, phenolics, saponins, and steroids and negative for terpenoid secondary metabolites

Table 2. Phytochemical analysis of methanolic plant extract

Plant	**Secondary metabolite**					
Baccaurea angulata	Alkaloid	Flavonoid	Saponin	Steroid	Tannin	Terpenoid
	+	+	+	+	+	–

The yield value of the methanol extract of *Baccaurea angulata* leaves is 4%, while the yield requirement is not less than 10%. According to (Fajarullah, 2015) a high yield value indicates the large number of bioactive components contained. Characterization of *Baccaurea angulata*, the yield value is still relatively low so that the bioactive components contained are still small (Ditjen POM, 2000).

Alkaloids can interfere with the integrity of the peptidoglycan component of bacterial cells, flavonoids can bring destruction upon the bacteria as it could cause the membrane to leak out cell material. Other studies had also documented that medicinal plants contain coumarins, flavonoids, phenolics, alkaloids, terpenoids, tannins, and polyacetylenes which have the potential as a bactericidal, bacteriostatic, or fungicidal effect against selected human pathogens [Motaleb, 2011; Dholaria, 2018.]

It is known that saponins are strong hemolytic agents and have soap-like properties. The function of saponins is as an antimicrobial, anti-inflammatory, and anti- cytotoxic.

3.2 Antimicrobial Activity Testing

Table 3 summarizes the average microbial growth inhibition of *Baccaurea angulata* leaves extract. This due to the sensitivity of the extract depends on the level of the concentration when it is used against certain microorganisms.

The in vitro antimicrobial activities of methanol extracts of *Baccaurea angulata* leaves concentrations of 40%, 60%, 80%, and 100% were tested against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. Chloramphenicol, Ketoconazole was used as a positive control because it is a broad-spectrum antibiotic and antifungal, and DMSO as a negative control. All the tests were performed in triplicates and were repeated three times.

The results measuring the inhibition zone for *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* had the largest inhibition zone at an extract concentration of 100%. This research shows that *Baccaurea angulata* leaves extract can inhibit the growth of *Staphylococcus aureus*, *Escherichia* coli bacteria, and *Candida albicans* fungus using the paper disc/Kirby-Bauer diffusion method.

The findings of the study may be helpful to future investigators in identifying alternative and new bioactive secondary metabolites like antimicrobial to treat resistant human pathogens

Table 3. Zone of Inhibition from Different Concentrations of *Baccaurea angulata* leaves

Extract/Control Conc. (%)	Zone of inhibition (mm)		
	Staphylococcus aureus ATCC 25923	***Escherichia coli ATCC 25922***	***Candida albicans ATCC 10231***
40	–	–	–
60	7.4	7.16	6.20
80	8.5	11.95	7.20
100	14.4	12.30	15.56
Chloramphenicol	20.2	30.6	–
Ketoconazole	–	–	18.36
DMSO	–	–	–

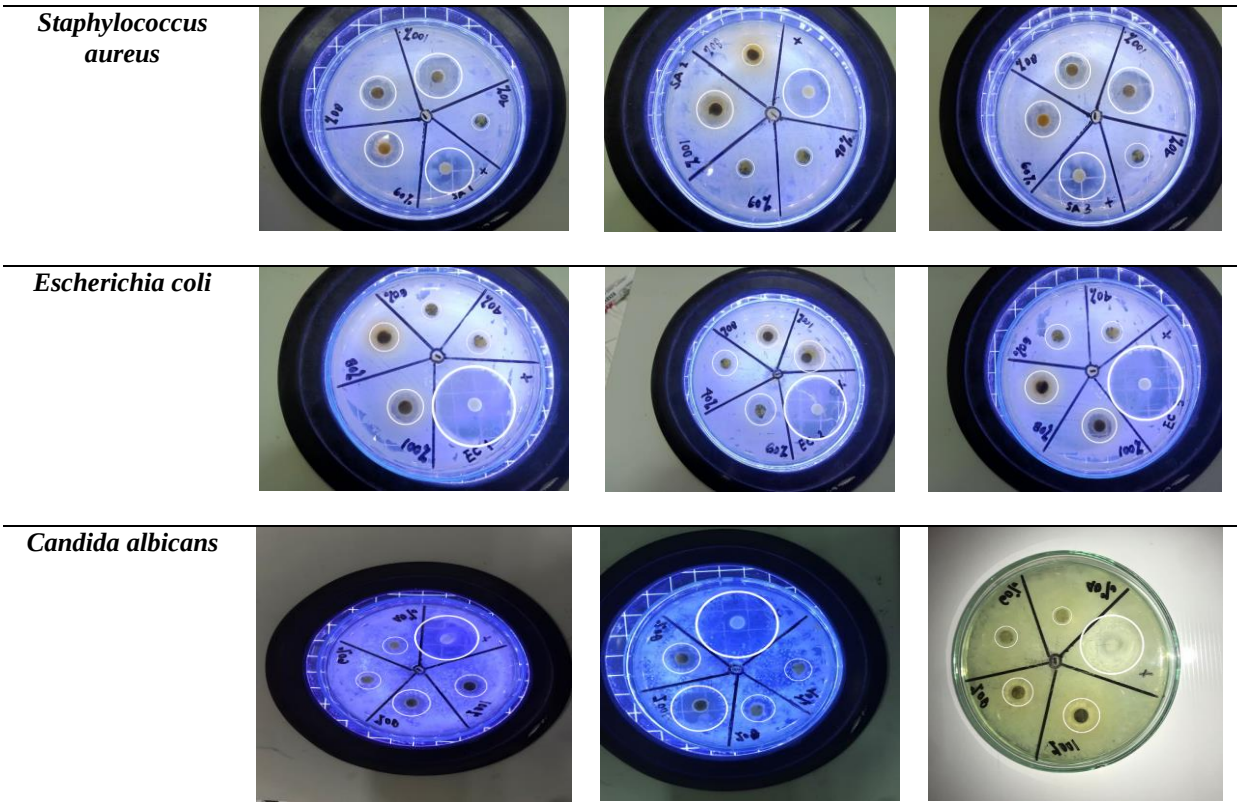

Figure 2. Antimicrobial activity of the *Baccaurea angulata* leaves extract

3.3. Toxicity of the *Baccaurea angulata* leaves extracts on brine shrimp larvae

Brine Shrimp Lethality Test (BSLT) is a screening method to determine the toxicity of an extract or natural compound. The BSLT method has been to correlate with anticancer activity. This method is easy to do, cheap, fast, and accurate (Meyer et al., 1982). The BSLT test aims to determine whether an extract can inhibit cell growth and undergo further procedures in the process of discovering anticancer drugs. The Brine Shrimp Lethality Test (BSLT) is a test using *Artemia salina* Leach test animals that can be used as a simple bioassay to examine the toxicity of a compound or sample by determining the LC50 value of the active components or extracts in plants (Moshi, M.J. et al., 2006).

BSLT using *Artemia salina* Leach shrimp larvae tested because they have high sensitivity to toxic compounds. The BSLT method is highly recommended for toxicity testing because it correlates up to a 95% confidence level with specific anticancer tests (Anderson, 1991: 107-111)

The toxicity test using the BSLT (Brine Shrimp Lethality Test) method can determine the number of deaths of *Artemia salina* Leach larvae, due to the influence of extracts or natural compounds at the given concentration (McLaughlin & Roger, 1998).

In the toxicity activity test, a compound has potential toxicity if the LC50 value is less than 1000 ppm. Lethal concentration 50 is the concentration at which a substance or sample can cause 50% death in experimental animals, namely *Artemia salina* Leach (Meyer et al., 1982). The concentrations used in toxicity activity testing are 50 ppm, 125 ppm, 250 ppm, 250 ppm, and 500 ppm.

In the toxicity test, the LC50 value for *Baccaurea angulata* leaves extract was 605.29 ppm in the moderate toxic category. The conclusion is that the methanol extract of *Baccaurea angulata* leaves has potential toxicity using the BSLT method, so that further research further testing can be carried out and developed as an anticancer drug (Carbollo, 2002)

Table 4 summarizes the mortality data of *Baccaurea angulata* leaves extract. This due to the sensitivity of the extract depends on the level of the concentration when it is used against certain microorganisms [9].

Tabel 4. Mortality data of *Baccaurea angulata* leaves extract

Conc. (ppm)	Log conc.	number of test larvae	number of dead larvae				% dead of larvae	Probit	LC 50 (ppm)
			1	2	3	average			
500	2.7	10	5	4	4	4.333	43.33	4.82	**605,29 (toxic)**
250	2.4	10	3	2	3	2.670	26.67	4..35	
125	2.1	10	2	1	0	1	10	3.72	
50	1.7	10	0	0	1	0.333	3.33	3.13	
0	0	10	0	0	0	0	0	0	

IV. CONCLUSION

Methanol extract of *Baccaurea angulata* leaves with a concentration of 100 % has antimicrobial activity, which can inhibit the growth of, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and Candida albicans ATCC 10231, and has toxicity activity shown at the LC50 value of 605.29 ppm with the moderate toxic category.

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