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Antibacterial Activity Against Gram-Positive and Gram-Negative Bacteria and Biolarvicide to *Aedes aegypti* from Cocok Bubu (*Elatostema rostratum* (Blume) Hassk) Leaves Extract

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Abstract

Tropical diseases are infectious diseases that commonly occur in tropical climates. Infectious diseases are caused by bacteria, viruses, parasites, and are transmitted by several vectors. There are 8 neglected tropical diseases (NTDs) that have spread in Indonesia. Therefore, massive efforts are needed to overcome this disease. Active plant substances have long been popular in treating various diseases. Cocok Bubu (*Elatostema rostratum* (Blume) Hassk) is an endemic plant of Indonesia. Ethnobotanical studies of this plant are used to treat fever and diarrhea. But, there are no pharmacological studies on previous research because this species belongs to a limited distribution plant. However, *Elatostema* has a secondary metabolite with various pharmacological activities. This study aims to determine the activity of antibacterial and biolarvicides from Cocok bubu leaf extract. The extraction using the maceration method with acetone, phytochemicals screening, toxicity test using BSLT, antibacterial test with disc diffusion to *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus mutant*, and *Streptococcus sanguinis*, and biolarvicide activity against *Aedes aegypti*. Based on the results, Cocok bubu leaf extract contains flavonoids, alkaloids, saponin, tannin, terpenoids, and steroids with a toxicity of 758.45 ppm. From antibacterial activity results, Cocok bubu exctract had the best inhibition zone against *Staphylococcus aureus* (10 – 18 mm). According to the biolarvicide test, it is effective as a biolarvicide to *Aedes aegypti* with LC_{50} 51.099 ppm and LT_{50} of 5 h 43 min. This study shows that Cocok bubu was more effective for treating and preventing NTDs in Indonesia, especially dengue and chikungunya fever in the future.

Keywords: *neglected tropical disease (NTDs), cocok bubu (elatostema rostratum (Blume) Hassk), antibacterial, biolarvicide.*

Introduction

Indonesia is a country located on the equator and is a place for the spread of tropical diseases, namely infectious diseases caused by bacteria, viruses, and parasites[1]. In addition, the infectious disease can be transmitted by vectors. For example, *Staphylococcus aureus* causes pneumonia, osteomyelitis, and endocarditis[2]. The most common vectors are mosquitos, such as *Culex sp.*, *Anopheles sp.*, and *Aedes sp.* These vectors cause filariasis, Japanese

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encephalitis, malaria, dengue hemorrhagic fever (DHF), chikungunya, and yellow fever[3]. In Indonesia, this infectious disease is included in the top 10 (28.1%) causes of death[4].

Moreover, there are eight neglected tropical diseases (NTDs) that have spread in Indonesia[5]. These diseases are lymphatic filariasis, schistosomiasis, trichuriasis, ascariasis, leprosy, yaws, dengue and chikungunya fever, scabies, and myiasis. Therefore, massive efforts from *stakeholders* are needed to overcome this disease. Active plant substances have long been excellent in treating various diseases. The discovery of new antibiotics from plants can begin by testing their antibacterial activity. Vector control can be carried out using biolarvicides, which are more eco-friendly and do not cause resistance.

In general, almost all NTDs appear to have the same symptoms, especially fever. According to Mackowiak et.al.[6], fever is an important component of a diagnostic screening program, particularly for infections, because it is the only disease complaint that is commonly reported by a wide range of patients. Cocok Bubu (*Elatostema rostratum* (Blume) Hassk.) is an endemic plant of Indonesia that belongs to Urticaceae. Ethnobotanical study on this medical plant in Sukabumi, many people use this plant to treat fever and diarrhea. However, there have been no pharmacological studies of this plant in previous research because this species has a limited distribution plant. It was distributed in Java, Sulawesi, and Sumatra.

Furthemore, *Elatostema* has pharmacological effects as an analgesic, anti-inflammatory, antioxidant, antibacterial, and antimicrobial[7]. Ethnopharmacology these plants can be used for hypertension, fever, asthma, kidney disease, rheumatoid arthritis, injuries, abdominal pain, boils, swelling, headache, stomachache, and diarrhea[8][9][10]. Ethnobotanical studies on *Elatostema* are utilized as food and medicine, such as for fever, immunodeficiency in children, liver disease, treating wounds, abdominal pain, joint pain, hypertension, injury, scabies, and headache [11].

Several studies of Elatostema have reported as antibacterial activity, such as those of *E. parasiticum, E. papillosum, E. repens, E. sinuatum, and E. integrifolium*[7][12][13][14]. In larvicidal activity, only one species has been reported (*E. sinnatum*), but the result is not active. However, *Elatostema* contains a various of secondary metabolites, i.e., phenolic compounds, flavonoid, alkaloid, terpenoid, and steroid[9][15]. Several compounds have been isolated from *Elatostema*, specifically 4,4',6'-trihydroxy-3-methoxy-3'-pentene chalcone, mushroom alcohol, quinoline, limonene, nerol, linalool, thymol, phytol, neopitadine, palmitic acid, and linoleic[9][12][16]. Some of these compounds have various biological activities, including antibacterial and biolarvicidal, which have been reviewed in the research of Farhadi et.al.[17], Yan et.al[18] and Wuillda et.al[19]. Cocok Bubu is predicted to have a similar secondary metabolite to this genus. Therefore, Cocok bubu is potential plant for treatment and prevention of such infectious disease, by using it as an antibacterial and antilarvae (biolarvicide).

Experimental

Materials

The materials used in this study were amoxicillin, acetone (90%, Bratachem), non-iodine salt, glacial acetic acid p.a (100%, Merck), chloroform p.a (100%, Merck), mercuric chloride p.a (100%, Merck), sulfuric acid chloride (98%, Merck), hydrochloric acid (37%, Merck), potassium iodide p.a (100%, Merck), magnesium powder (Merck), ferric chloride (100%, Merck), and dimethyl sulfoxide (Merck), *nutrient agar* (Merck), *nutrient broth* (Merck), and bacterial cultures of *Escherichia coli ATCC 11229, Staphylococcus aureus ATCC 6538, Streptococcus mutans ATCC 25175,* and *Streptococcus sanguinis ATCC 10556.* The leaves of Cocok Bubu (*E.* rostratum (Blume) Hassk.) were obtained from Sukaraja area, Sukabumi district, West Java, Indonesia. The plant was botanically identified by the Center for Biological Research-LIPI (Indonesian Institute of Sciences) with No. 715/IPH.1.01 /If.07/IV/2019.

Instruments

The Instrument used consisted of maceration equipment, vacuum pump, analytical balance (AND HR-250AZ, Japan), Buchner funnels, vacuum rotary evaporator (IKA RV 10 Basic V, Germany), water bath (JULABO SW-20C/3, Germany), autoclave, and incubator.

Methods

Extraction

Cocok Bubu leaves were cleaned, dried, and mashed as simplicia. After that, 1000g of simplicia was extracted using the maceration method with acetone solvent for 24 h, repeated three times.

Phytochemical Screening

The phytochemical screening from Cocok bubu leave extract consisted of testing for tannin, saponin, steroid, triterpenoid, flavonoid, alkaloid, and terpenoid by Harbone, following the procedures of Adusei et.al.[20] and Iqbal et.al.[21].

Toxicity Test

The Brine Shrimp Lethality Test (BSLT) method used for toxicity testing was conducted following the method as previously described[22]. A total of 10 healthy shrimp larvae aged 48 h were put into a test tube containing synthetic salt solution (20 g of non-iodine salt in 1 l of distilled water), then Cocok Bubu extract solution was added in each test tube with 5 concentration variations, namely 10, 20, 30, 40, and 50 mg/l, and observation was made after 24 h by counting the number of dead larvae.

Antibacterial Test

Antibacterial testing was analyzed using the agar disk-diffusion method with treatments that have been adapted to the requirements of the research[23]. 30 µL of Cocok Bubu leaf extract with concentrations of 20, 50, and 100% was added into Petri dish containing nutrient agar which had previously been suspended by bacteria *E. coli, S. aureus, S. mutans, S. sanguinis* and incubated for 1x24 h. The positive control used was amoxicillin 100 mg/l and the negative control was DMSO 10%.

Biolarvicide Test

The test in determining the activity of biolarvicide is bioassay method with the addition of a slight modification[24]. 20 *Aedes aegypti* larvae were put in beakers containing 200 ml Cocok Bubu extract solution, containing 10, 50, 100, and 250 mg/l respectively. Observation of the dead larvae was done after 24 h of contact time.

Data Analysis

The data from toxicity and biolarvicide activity test results were analyzed statistically with SPSS version 25 software.

Results and Discussion

Phytochemical Screening of *E. rostratum* leave extract

The results of the phytochemical screening of Cocok Bubu leaf extract were positive for alkaloid, saponin, flavonoid, tannin, terpenoid, and steroid. These results are consistent with those reported by Uddin et.al.[7], Yin et.al.[9], Reza et.al.[15], Miyazawa et.al.[16], who have stated that in these species there are compounds from the flavonoid, phenolic, essential oil, triterpenoid, steroid, and terpenoid. Phytochemical screening underlies the potential of Cocok Bubu leaf extract as an antibacterial and biolarvicide.

Toxicity Test of *E. rostratum* leave extract

The first toxicity test used the BSLT method (Brine Shrimp Lethality Test). This is a simple test that is commonly used for toxicity evaluation, and this analysis is often used for environmental problems. The toxicity test of Cocok Bubu leaf extract resulted in a LC_{50} value of 798.82 mg/L. A substance or material with an LC_{50} value <1000 mg/l has the potential for bioactivities, including as an antibacterial and larvacide[25]. Research conducted by Purnama[26] and Rahmawati[27] on the *Smilax leucophylla*

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Blume plant with toxicity value of 758.45 mg/L that have the two activities. Other plants that have the same activities are *Jatropha curcas* and *Ficus religiosa* as antibacterial and larvacide[28][29].

Antibacterial Activity of *E. rostratum* Leave Extract

Results of an antibacterial test of Cocok Bubu leaf extract against *E. coli, S. aureus, S. mutants*, and *S. sanguinis* are presented in Table 1 and figure 1.

Data presented in Table 1 displays that Cocok Bubu leaf extract has no inhibitory or antibacterial activity against *S. mutans* and *E. coli*, the average value of a zone of inhibition <10mm. According to Greenwood, a compound or a sample is categorized active as antibacterial if the average value of the inhibition zone diameter is >20 mm, the sample has strong inhibitory power, a value of 16-20 mm is moderate, a value of 10-15 mm is weak and if the value is <10 mm it is categorized as lacking inhibitory power [30]. Cocok Bubu leaf extract has the potential to be antibacterial against *S. sanguinis* at the highest concentration. On the contrary, the antibacterial activity of Cocok Bubu leaf extract against *S. aureus* has shown a much better zone of inhibition, the zone of inhibition is in the range of 10-20mm. The potential of *E. rostratum* as an antibacterial against *S. aureus* is in accordance with that reported by Uddin et.al., [7] and Mariani et.al., [13] on another *Elatostema* species.

Table 1. The antibacterial activities of Cocok Bubu leaf extract

Description	Zone of inhibition (mm)			
	E. coli	*S. aureus*	*S. mutants*	*S. sanguinis*
Control (+)	11.90±0.50	30.68±0.07	11.85±0.65	10.38±0.07
Control (-)	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
Extract 100%	6.70±0.70*	17.58±1.23	6.00±0.00	12.10±0.35*
Extract 50%	6.00±0.00	13.48±0.78	6.00±0.00	6.00±0.00
Extract 20%	6.00±0.00	9.90±0.75	6.00±0.00	6.00±0.00

*zone of inhibition is not clearing

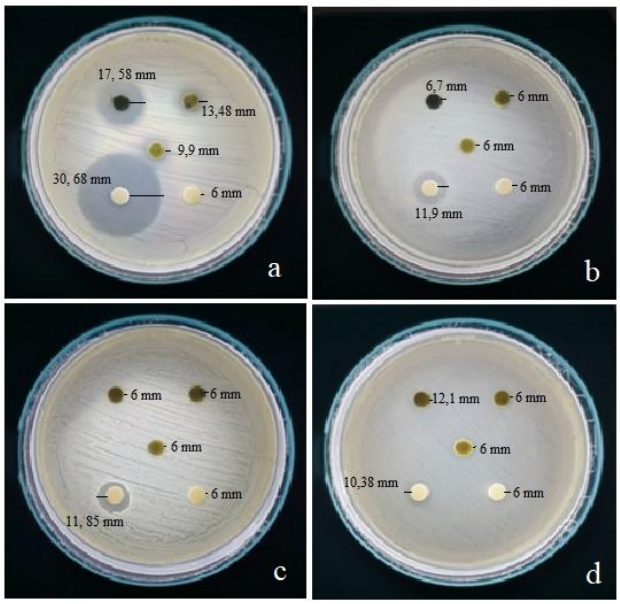

Figure 1. The antibacterial test results of *Cocok Bubu* leaf extract on a) *S. aureus;* b) *E. coli;* c) *S. mutans;* d) *S. sanguinis.*

The effectiveness of Cocok Bubu leaf extract as an antibacterial against *S. aureus* has been reported by other researchers because it has a bacteriostatic and bactericidal nature due to the unique contents of alkaloid, saponin, flavonoid, tannin, terpenoid, and steroid compounds; thus, it only has an antibacterial activity that is selective against these bacteria with a different mechanism of action, according to Khameneh et.al. [31], the target of a compound as an antibacterial in general is to disrupt the biosynthesis of proteins, cell walls, bacterial membranes, bacterial metabolic pathways, and DNA replication. One example of the mechanism of action carried out by flavonoid, tannin, terpenoid, and alkaloid compounds is as an inhibitor of the FtsZ (Filamenting temperature-sensitive mutant Z) protein with various interactions[32].

It is a different case with the saponin and steroid groups, which are predicted to inhibit the working system of the cell membranes by increasing membrane permeability or inhibiting sortase protein which can cause the membrane to experience lysis [33][34].

Some of the compounds belong the to flavonoid, terpenoid, polyphenol and alkaloid found in plants of the genus *Elatostema* and the Urticaceae family include chalcone, quinoline, kaempferol, catechins, thymol, vanillin, kinkonaine, and bohmerone which are thought to be also found in Cocok Bubu leaf extract [35]. These compounds have been reported to have antibacterial activity, particularly against *S. aureus*[17][36][37][38].

Biolarvicide

The data is shown in Figure 3. The effect of the Cocok Bubu leaf extract concentrations on the mortality percentage of *Aedes aegypti* larvae.

The high concentration of Cocok Bubu leaf extract resulted in the high mortality rate of the test larvae. The concentration of Cocok Bubu leaf extract which has caused a 100% mortality rate of *Aedes aegypti* larvae, is 100 mg/L. As a result, Cocok Bubu leaf extract is effective as a biolarvicide against Aedes aegypti according to the WHO biolarvicide standards [24]. Furthermore, based on Figure 2, the LC_{50} value of Cocok Bubu leaf extract is 51.099 mg/l. This result is different from that of the research conducted by Alvarez et.al.[39], who stated that the ethanol extract of *Elatostema sinnatum* did not show larvicidal activity against *Aedes aegypti*. This can happen because different species cause different content of secondary metabolites, especially related to compounds that are active as larvicides. This difference is caused by differences in geographic location and an environmental conditions in which it grows, including nutrients in the soil, water content, and soil pH.

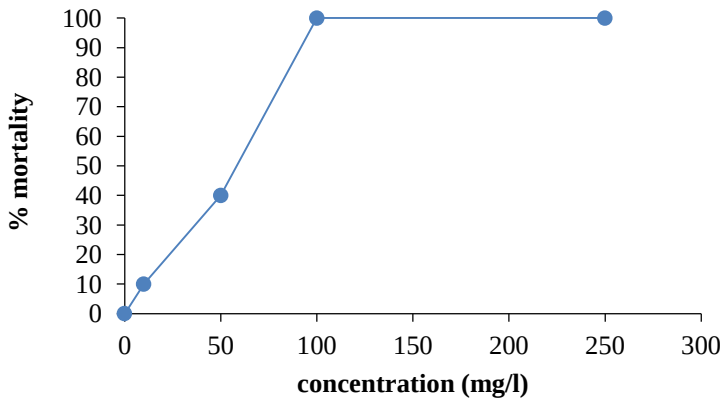

Figure 2. Effect of Cocok Bubu leaf extract concentrations on the mortality percentage of *Aedes aegypti* larvae for 24 h

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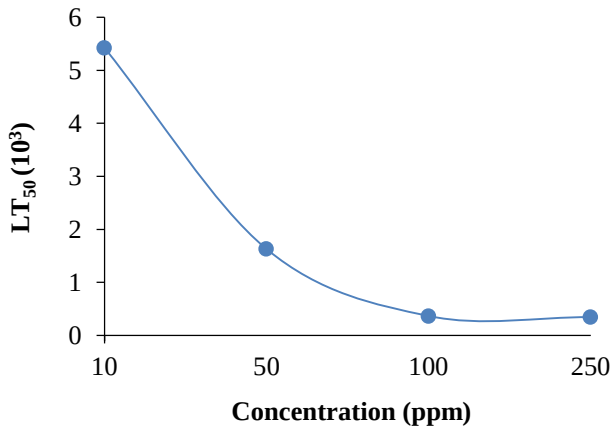

Figure 3. The relationship between the concentration of Cocok Bubu leaf extract and LT_{50}

Another parameter for the biolarvicide toxicity test was LT_{50}. The relationship between the concentration of Cocok Bubu leaf extract and LT_{50} is presented Figure 3.

A high concentration of Cocok Bubu causes the low value of LT_{50}. This indicates that the mortality rate of larvae is faster. At a concentration of 10 ppm, the time needed to kill 50% of the population of test larvae is about 3 d 18 h, and at the concentrations of 50, 100, and 250 ppm, the times taken to kill the larvae are respectively 27 h, 6 h 5 min, and 5 h 43 min.

The presence of larvicidal activity is supported by the presence of secondary metabolites of alkaloid, flavonoid, tannin, saponin, steroid, and terpenoid in Cocok Bubu leaf extract. The mechanism of alkaloids in inhibiting or killing the larvae can occur in a variety of ways, but mostly affecting the acetylcholine receptor (ACh) in the nervous system.

Cholinesterase (AChE) is a family of enzymes that catalyze the hydrolysis of acetylcholine (ACh) neurotransmitters to choline and acetic acid. The alkaloid in the AChE substrate is the reason for the hydrolysis reaction in larvae metabolism not to occur. This prevents the formation of choline and acetic acid which will result in the brittle structure of the larvae and the non-optimal work of the body's cells [40].

Another group of secondary metabolites that has the potential as biolarvicide in Cocok Bubu leaf extract is a flavonoid. The mechanism of flavonoids in inhibiting or killing larvae occurs in several ways; such as acting as a mitochondrial poison by inhibiting the chain reaction of electron transport, hence it cannot produce energy[41]. Based on the results of phytochemical screening, flavone is a type of flavonoid present in Cocok Bubu. In Addition, the same genus and family also found similar compounds. Therefore, the mechanism of flavonoids from Cocok Bubu leaf extract that function as biolarvicide is through interaction with the active side of proteins in sterol carrier protein-2 (SCP-2), where these proteins are carriers of sterols which are nutrients for larvae[42].

Cocok Bubu as a biolarvicide is also supported by the presence of tannin. Tannin causes peritrophic membrane extrusion, in which this membrane protects the midgut epithelium of the larvae from chemicals, toxins, pathogens, and mechanical damage. This causes the peritrophic membrane to be[43]. The larvicidal activity of Cocok Bubu leaf extract can also be influenced by the presence of saponin. Saponin compounds have lipophilic components that easily interact with the epicutela lipid layer and disrupt the endocuticular protein layer in the plasma membrane. This interaction can disrupt the plasma membrane, causing damage to its structure and function[44].

Steroid activity in influencing the larvae has not been widely reported. However, the steroid is a growth hormone that affects skin turnover in larvae. The addition of steroid that comes from the outside can affect the thickening of the cell walls in the chitin, thus the larvae become abnormal[45]. The last secondary metabolite with biolarvicidal activity is terpene or terpenoid. The inhibition process is caused by the presence of the interaction between terpene or terpenoid and amino acid residue in protein (SCP-2). It was reported that the groups of this compound that are active as larvicides, in general, are monoterpene and monoterpenoid, for example isopulegol, lavandulol, limonen, thymol, linalol, menthol, menton, mirsen, and neoisopulegol[46]. From the compounds, which are found in Elatostema are limonene and thymol[9]. Overall, the mechanism of action of secondary metabolites in Cocok Bubu leaf extract in killing larvae has many similarities. Therefore, in this case, the compounds contained in Cocok Bubu leaf extract are possibly working synergistically in their activity as biolarvicides. This is evidenced by the low value of LC_{50} of Cocok Bubu extract on *Aedes aegypti.*

Conclusions

Cocok Bubu (*Elatostema rostratum* (Blume) Hassk) leave extract have biolarvicidal potential against *Aedes aegypti* with high toxicity and is most effective as an antibacterial against *Staphylococcus aureus* with weak to moderate of inhibition zones range. Therefore, Cocok bubu is more potential for treatment and prevention of DHF and chikungunya, 2 from 8 of NTDs in Indonesia.

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