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Antibacterial Activity of Triterpenoid from the Leaves of Tahi Ayam (*Lantana camara* Linn.)

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Abstract

Tahi Ayam (*Lantana camara* Linn.) is known for its traditional medicinal purposes, can be used for treating various diseases. This is ascribed to the abundant presence of secondary metabolite compounds in *Lantana camara* Linn. In particular, the ethyl acetate extract of *Lantana camara* Linn. leaves contain secondary metabolite compounds belonging to the triterpenoid, steroid, coumarin, and phenolic groups. One of the triterpenoid compounds isolated from the ethyl acetate extract of *Lantana camara* Linn. leaves are 9-hydroxy-Lantadene A. Subsequently, an antibacterial test was conducted on this compound to assess its ability to inhibit the growth of test bacteria i.e. *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative). Amoxicillin was employed as a positive control. In addition to testing the antibacterial properties of the 9-hydroxy-Lantadene A compound, the ethyl acetate fraction of *Lantana camara* Linn. leaves also underwent the same antibacterial testing procedure. The results of this study suggest that both the isolated compound and the ethyl acetate fraction from *Lantana camara* Linn. leaves possess antibacterial activity. However, it's important to note that the antibacterial activity observed in both samples is relatively low. The possible cause of this could be the low sample concentrations utilized in the research.

Keywords: Tahi ayam (Lantana camara Linn.*), Triterpenoid, antibacterial*

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Abstrak (Indonesian)

Tahi Ayam (*Lantana camara* Linn.) dikenal sebagai tanaman herbal untuk beberapa penyakit dan telah digunakan dalam pengobatan tradisional. Hal ini karena *Lantana camara* Linn. mempunyai kandungan senyawa metabolit sekunder yang banyak. Pada ektrak etil asetat daun *Lantana camara* Linn. terdapat senyawa metabolit sekunder golongan triterpenoid, steroid, kumarin, dan fenolik. Salah satu senyawa golongan triterpenoid yang berhasil diisolasi dari ektrak etil asetat daun *Lantana camara* Linn. adalah senyawa 9-hidroksi-Lantadena A. Uji antibakteri dilakukan terhadap senyawa tersebut untuk mengetahui kemampuannya dalam menghambat pertumbuhan bakteri uji. Bakteri uji yang digunakan adalah *Staphylococcus aureus* sebagai Gram positif dan *Escherichia coli* sebagai Gram negatif serta sebagai kontrol positif digunakan amoksilin yang sangat resisten terhadap *Staphylococcus aureus* dan *Escherichia coli*. Selain uji antibakteri terhadap senyawa 9-hidroksi-Lantadena A , uji antibakteri juga dilakukan terhadap fraksi etil asetat daun *Lantana camara* Linn. dengan pengerjaan yang sama. Hasil penelitian ini menunjukkan bahwa senyawa hasil isolasi dan fraksi etil asetat daun *Lantana camara* Linn. memiliki aktivitas antibakteri. Namun aktivitas antibakteri kedua sampel menunjukkan aktivitas yang rendah. Hal ini dikarenakan konsentrasi sampel yang digunakan sangat kecil.

Kata Kunci: Tahi ayam (Lantana camara Linn), Triterpenoid, antibakteri

INTRODUCTION

Lantana camara Linn plant in taxonomic classification belong to the Verbenaceae Family, *Lantana* Genus, and *camara* Species [1]. *Lantana camara* Linn. is a shrub, easy to grow and easy to find. This plant has been traditionally used to treat various diseases, including skin and digestive disorders, tetanus, malaria, tumors, rheumatic drugs, wounds, fever, asthma, chickenpox, cancer, boils, eczema, and heart disease. *Lantana camara* Linn. plant has variety of bioactivities that have been reported, such as antimicrobial, antibacterial, anxiolytic, antidiabetic, insecticidal, inhibitors of DNA damage, cytotoxic activity, anti-inflammatory, antioxidant, and phytopathogenic control on fusarium species [2-16].

Based on phytochemical screening, the methanol extract contains alkaloids, flavonoids, tannins, glycosides, carbohydrates, and triterpenoids, and ethyl acetate extract contains triterpenoids, steroids, coumarins, and phenolics [1,12]. Meanwhile, the reported compounds of this plant include Lantadene B, glycoside flavonoid, 22*β*-acetoxy-4-epi-hederagonic acid, 3-oxo-12α,22β,25-trihydroxy-18β-olean-28,13β-olide [17-19].

In the previous study, a triterpenoid compound, namely 9-hydroxy-Lantadene A, was successfully obtained. The compound was isolated from the ethyl acetate fraction of the leaves of the *Lantana camara* Linn. (**Figure** 1) [20].

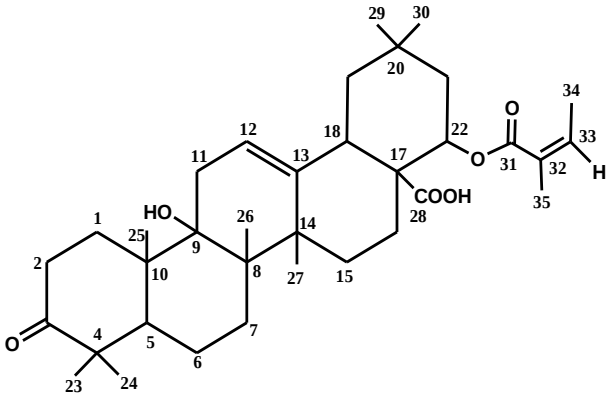

Figure 1. The structure of the isolated compound

MATERIALS AND METHODS

Materials

Material used in this research is magnetic stirrer (Corning PC-42000), magnetic bar, incubator (Gallenkamp), test tube, autoclave, petri dish, micropipette (Nichipet Ex), ose needle, spiritus, gauze, cotton, laminar air flow (Aneka Lab H.S 079S). Gram positive bacteria (*S. aureus*) and Gram-negative bacteria (*E. coli*), amoxicillin, Mueller-Hinton agar media, Nutrient Agar media, disc paper and alcohol

Bacterial inoculation

2 g of nutrient agar (NA) media was added into a 250 mL Erlenmeyer flask, followed by the addition of 100 mL of distilled water. The mixture was subsequently heated and stirred utilizing a magnetic stirrer until complete dissolution and boiling were achieved [21]. Meanwhile, gauze and cotton were used to cover the inoculating loop and test tube, which were then enveloped in aluminum foil. The prepared Erlenmeyer flask containing the media, along with the covered test tube and inoculating loop, was carefully positioned within the autoclave. Sterilization was carried out at a pressure of 15 lbs and a temperature of 121°C for a duration of 15 minutes.

The NA media was poured into a test tube (filled to approximately one-third of its capacity) and allowed to solidify. After solidification, bacterial cultures of *E. coli* and *S. aureus* were aseptically inoculated onto the media using an inoculating loop. The inoculated test tubes were then incubated at 37°C for 24 hours within an incubator

Preparation of Mueller-Hinton Media

7.2 grams of Mueller-Hinton agar (MHA) media was added to 200 mL of distilled water. The solution was subsequently heated and stirred utilizing a magnetic stirrer until complete dissolution and boiling were achieved [22]. Then, the MHA solution underwent sterilization through autoclaving under a pressure of 15 lbs and at a temperature of 121°C. Following sterilization, 15 mL of the prepared MHA solution was poured into a sterile petri dish and allowed to solidify within the confines of a laminar flow cabinet.

Preparation of Test Solutions

10 mg of each sample (the ethyl acetate fraction and the isolated compound were weighed and dissolved in a 10 mL flask with ethyl acetate up to the mark and made with several variations of concentration (1000, 500, 250, 125, and 62.5 µg/mL).

Antibacterial Activity Testing

Test bacteria (200 µL) were poured into MHA and left to dry for 15 minutes at room temperature in laminar airflow. Sterile paper discs were watered with the test solution (10 µL) for each concentration. These discs were then positioned onto MHA media and incubated at room temperature for 24 hours. The experiment was carried out two times (Duplo). In this experiment, the positive control used amoxicillin 62.5 µg/mL, and the negative control used ethyl acetate distillate. The presence of the inhibition zone around the disc indicates an area of inhibition of the growth

of the test bacteria. The diameter of the inhibition zone was measured horizontally and vertically using a measuring instrument [23].

RESULTS AND DISCUSSION

Antibacterial Activity

The antibacterial activity from the ethyl acetate fraction of *Lantana camara* Linn. leaves and the isolated compound was using the disk diffusion method against *Staphylococcus aureus* and *Escherichia coli*. These bacteria were selected based on the traditional use of *Lantana camara* Linn. leaves as a medicine for skin diseases and digestive disorders. *Staphylococcus aureus* is a bacterium that commonly grows and develops in skin tissue, while *Escherichia coli* grows and develops in the digestive tract. Moreover, both bacteria are widely used as test organisms to represent gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria in antibacterial testing [24-26].

Based on **Table** 1, it is evident that the isolated triterpenoid compound and the ethyl acetate fraction exhibit antibacterial activity, as indicated by the presence of inhibition zones around the discs at concentrations of 10 µg/disk and 5 µg/disk. However, concentrations ranging from 2.5 µg/disk to 0.625 µg/disk did not show such antibacterial effects. The data implies that the isolated compound and the ethyl acetate fraction can effectively function as antibacterial agents at ≥ 5 µg/disk.

Based on the CLSI data (Clinical and Laboratory Standards Institute), the sample's antibacterial efficacy is determined by comparing the inhibition zone diameter samples to the inhibition zone diameter of the positive control. Each antibacterial positive control has a specific range for each level of antibacterial activity (strong, moderate, and weak). In this study, amoxicillin was used as the positive control, allowing the assessment of antibacterial activity levels as follows: strong if the inhibition zone diameter is greater than or equal to 18 mm, moderate (14 – 17 mm), and weak (≤13 mm) for amoxicillin 10µg [27]. Based on the inhibition zone measurement results for both bacterial strains, it is evident that the isolated triterpenoid compound and the ethyl acetate fraction exhibit antibacterial properties, indicated by the presence of clear zones around the discs at concentrations of 10 µg/disk and 5 µg/disk. However, the antibacterial efficacy of the isolated triterpenoid compound and the ethyl acetate fraction is weak for 10 µg/disk and 5 µg/disk concentrations.

Table 1. The result of inhibition zone measurements

Test Solutions	Conc. (µg)	Diameter of the inhibition zone (mm)	
		E. coli	*S. aureus*
Ethyl acetate fraction	10	1	2
	5	1	1
	2.5	-	-
	1.25	-	-
	0.625	-	-
Isolated triterpenoid compound	10	1	1
	5	1	1
	2.5	-	-
	1.25	-	-
	0.625	-	-
Control (+) a*moxicillin*	0.625	3	3
Control (-) ethyl acetate distillate	-	-	-

Based on the antibacterial test results of the ethyl acetate extract reported at different concentrations (50 mg/ml, 100 mg/ml, and 150 mg/ml) for both *S. aureus* and *E. coli*. 50 mg/ml concentration, the inhibition zones for *S. aureus* and *E. coli* were 15.00 mm and 7.83 mm, respectively. Increasing the concentration to 100 mg/ml resulted in larger inhibition zones, measuring 17.33 mm for *S. aureus* and 12.50 mm for *E. coli*. The highest concentration tested, 150 mg/ml, exhibited even greater inhibition zones of 18.00 mm for *S. aureus* and 15.17 mm for *E. coli* [28]. Comparing these results to the research finding, it is evident that the ethyl acetate extract exhibited stronger antibacterial activity against *S. aureus* when compared to *E. coli*.

CONCLUSION

Test antibacterial activity has been done on the ethyl acetate extract and the isolated compound from the ethyl acetate extract of *Lantana camara* Linn. The test results indicate that both samples exhibited weak antibacterial activity at 5 and 10 µg/disk concentrations. Based on their ability to inhibit the growth of the test bacteria, the ethyl acetate extract indicated stronger activity against *S. aureus* compared to *E. coli*.

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