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Antibacterial Activity of Lidah Mertua (*Sansevieria Trifasciata* Prain.) Leaves Extract on *Escherichia coli* and *Staphylococcus aureus*

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Abstract

BACKGROUND: Infection is the most common diseases in developing country, including Indonesia. Bacteria that often causes infection is *Escherichia coli* and *Staphylococcus aureus.* One of the traditional plants that can be used as an antibacterial is lidah mertua.

AIM: The purpose of this study was to find out the profile of chemical compounds by thin layer chromatography method and determine the antibacterial activity of Lidah Mertua leaves by in vitro.

METHODS: This research conducted an experimentally using non-polar, semipolar, and polar as solvents to get extract against *E. coli* and *S. aureus* as bacterial testing. The antibacterial activity using agar diffusion method to get minimum inhibitory concentration (MIC).

RESULTS: The result of the research on thin layer chromatography showed that the compounds contained in the Lidah Mertua leaves were polifenol, steroids and alkaloids. The data obtained were tabulated and analysed descriptively. The antibacterial activity show that n-hexane extract does not provide inhibitory activity. MIC value show that aethyl acetate extract of lidah mertua leaves inhibited the growth of *E. coli* and *S. aureus* at concentration 50 mg/mL and 25 mg/mL with diameters of inhibition zone is 8.50 mm and 8.20 mm and methanol extract of lidah mertua leaves inhibited the growth of *E. coli* and *S. aureus* at concentration 12.5 mg/mL and 25 mg/mL with diameters of inhibition zone is 8.46 mm and 8.32 mm.

CONCLUSION: The profile of chemical compounds by thin layer chromatography method showed that the compounds contained in the Lidah Mertua leaves were polifenol, steroids and alkaloids. The antibacterial activity show that n-hexane extract does not provide inhibitory activity, but aethyl acetate extract of lidah mertua leaves inhibited the growth of *E. coli* and *S. aureus.*

Introduction

Infectious diseases are the most suffered by people in developing countries, including Indonesia. One of the causes of infectious diseases is bacteria. Bacteria are microorganisms that cannot be seen with ordinary eyes, but can only be seen with the aid of a microscope. Bacteria that often cause infections in humans include *Staphylococcus aureus* and *Escherichia coli*. *Staphylococcus aureus* infection can hemolysis the blood and plasma, and can cause difficult treatment problems. Bacteria that cause respiratory and gastrointestinal infections are *Escherichia coli* [1].

One antibacterial substance that is widely used is antibiotics. Antibiotics are typical chemical compounds produced or derived by living organisms including their analogue structures made synthetically, which in low levels can inhibit important processes in the life of one or more species of microorganisms [2]. In the treatment of antibacterial infections other alternatives can be used by using herbal medicines that can minimize side effects [3]. One of them is the *leaves* of the *lidah mertua* (*Sansevieria trifasciata* Prain.).

Lidah mertua (*Sansevieria trifasciata* Prain.) Is an ornamental plant that is easy to grow on the home page without much care. The benefits of Lidah Mertua leaves can be used to treat colds, diarrhoea, cough, inflammation of the respiratory tract (bronchitis), swelling due to bumps (bruises), ulcers, ulcers, bites of poisonous snakes and hair fertilisers.

Leaves content *Lidah mertua* consists of abamagenin, kardenolin, saponins and polyphenols [4].

N-hexane solvents are a type of nonpolar solvent so that n-hexane can dissolve nonpolar compounds [5]. Ethyl acetate is a semi-polar solvent and can dissolve semi-polar compounds in the cell wall [6]. According to Kusumaningtyas et al., (2008) [7] methanol is a polar solvent that can dissolve polar compounds such as phenol groups. Based on the description above, the authors are interested in researching the antibacterial activity of *lidah mertualeaves* extract (*Sansevieria trifasciata* Prain) with the growth of *Staphylococcus aureus* and *Escherichia coli*.

Material and Methods

Research has been carried out at the Laboratory of Microbiology, Faculty of Pharmacy in University of Tjut Nyak Dhien Medan from April to August 2018. Extraction of plant material was carried out by purposive sampling, the sample used was the lidah mertua (*Sansevieria trifasciata* Prain.) leaves, Obtained from Jalan Jawa, Medan Helvetia, Medan City, North Sumatra Province.

The tools used in this research are laboratory glassware (Pyrex®), Blender (Philips®), incubator (Memert®), caliper, gas stove (Rinnai®), refrigerators (Panasonic®), dryer cabinets, balance sheets analytic (Vibra AJ®), oven (Memert®), metal field, capillary pipe and water bath. The chemicals used unless otherwise stated are quality analysis pro (pa), distilled water, glacial acetic acid, hydrochloric acid, concentrated sulfuric acid, acetic acid anhydride, barium chloride, iron (III) chloride, bismuth (III) nitrate, dimethyl sulfoxide (DMSO), lidah mertua (*Sansevieria trifasciata* Prain.) leaves, Ethanol, ethylacetate, potassium iodide, chloroform, Macfarland, methanol, Mueller Hinton Agar media (merck®), Mueller Hinton Broth, Nutrient Agar (merck®), *n*-hexane and silica gel 60 F254. Bacteria test *Escherichia coli* and *Staphylococcus aureus*.

The research was carried out descriptively and experimentally by testing the extract of the leaves of the lidah mertua using non-polar, semipolar and polar as solvents for extraction method against the growth of *Staphylococcus aureus* and *Escherichia coli* as bacteria testing and the determination of extract chemical compounds by thin layer chromatography method. Antibacterial activity was tested using the diffusion agar method.

Results

Results of Plant Identification

The result of plant identification was carried out at the Laboratory of Herbarium Medanense (MEDA) University of North Sumatra, University of Sumatera Utara Medan, showing that the plant studied was Lidah Mertua (*Sansevieria trifasciata* Prain.) of Agavaceae family.

Results of The Acquisition of Simplicia

In this study, simplicia processing results using ± 6 kg of fresh lidah mertua leaves were dried in a drying cabinet, the leaves of the lidah mertua were considered dry when they were brittle (crushed into crushed), then blended to become powder. The weight of the simplicia powder obtained 600 grams.

Results of Extraction

The simplicia powder extraction process was made in multilevel maceration starting with *n*-hexane, aethyl acetate and methanol. Extraction results obtained with n-hexane solvent were 5.6 grams, ethyl acetate 3.7 grams and methanol produced 27.30 grams of dried blackish green extract.

n-Hexane is a type of nonpolar solvent so that n-hexane can dissolve nonpolar compounds [5]. Ethyl acetate is a semi-polar solvent and can dissolve semi-polar compounds in the cell wall [6]. According to [7] methanol is a polar solvent that can dissolve polar compounds such as phenol groups.

Results of Examination of Lidah Mertua Leaves Chemical Compounds with Thin Layer Chromatography (TLC)

Thin Layer Chromatography Test (TLC) aims to determine the chemical compounds in the extract of the Lidah Mertua leaves (*Sansevieria trifasciata* Prain.). Before filling the stationary sample, it must be activated by heating it in an oven at 110°C for 15 minutes. This is intended to increase the absorption power from increasing the absorption power of the stationary phase [8].

The stationary phase used in this research is silica gel 60 F_{254} plate with the mobile phase *n*-hexane: aethyl acetate (7: 3) for *n*-hexane and ethyl acetate extract while for methanol extract chloroform: aethyl acetate (3: 7) because it can provide more and more explicit stains to extract. The stain appearance used was 50% H_2SO_4 in methanol, FeCl3 5%, Liebermann-Burchard and Dragendroft.

Table 1: Results of TLC Chromatogram of n-hexane Extract of Lidah Mertua Leaves

Mobile phase: *n*-hexane-aethyl acetate (7: 3)	Visual	H_2SO_4 50% in methanol	$FeCl_3$ 5%	Lieberman-Bourchard	Dragendroff
n-hexane Extract	-	0.97(k) 0.93 (h) 0.91 (h) 0.88 (hk) 0.86 (hk) 0.80 (hk) 0.76 (h) 0.68 (h) 0.62 (h) 0.55 (h) 0.45 (h) 0.37 (h) 0.32 (h) 0.30 (h) 0.32 (k) 0.11 (h)	0.92 (h) 0.25 (h)	0.90 (bk) 0.78 (bk) 0.68 (bk) 0.30 (bk)	0.95 (c)

Note: (k): yellow; (h): green; (hk): blackish green; (c): brown; (bk): tosca.

The results of the TLC analyse of n-hexane extract with the mobile phase n-hexane: aethyl acetate (7: 3) using 50% H_2SO_4 in methanol as visualization gave 16 spots, namely green, yellow, blackish green, this indicates the presence of alkaloid compounds, polyphenols. The appearance of blemishes on Dragendorff as visualization gives brown colour as one spot, indicating the presence of alkaloid compounds. $FeCL_3$ 5% gives two green colours as spots indicating the presence of polyphenol group compounds. The appearance of Lieberman-Bourchard gives four spots of green-blue colored stains which means that the leaves of the *lidah mertua* (*Sansevieria trifasciata* Prain) have steroid compounds.

Table 2: Results of TLC Chromatogram of Ethylacetate Extract of Lidah mertua

Mobile phase: *n*-hexane-aethyl acetate (7: 3)	Visual	H_2SO_4 50% in methanol	$FeCl_3$ 5%	Lieberman-Bourchard	Dragendroff
Ethyl acetate extract	-	0.95 (kl) 0.86 (h) 0.79 (hl) 0.72 (ht) 0.61 (h) 0.18 (h) 0.52 (hk) 0.42 (h) 0.35 (ht) 0.31 (h) 0.27 (k) 0.23 (hl) 0.19 (k) 0.16 (h) 0.15 (h) 0.12 (h) 0.11 (k) 0.07 (h) 0.05 (h) 0.02 (h)	0.95 (h) 0.86 (h) 0.78 (hk) 0.72 (hk) 0.61(h) 0.42 (hk) 0.29 (h) 0.19 (b) 0.11 (hk) 0.06 (h)	0.79 (bkl) 0.71 (bk) 0.66 (bk)	0.61 (c) 0.05 (c)

Note: (b): blue; (c): brown; (h): green; (hl): weak green; (hk): blackish green; (ht): dark green; (k): yellow; (kl): weak yellow; (bk): tosca; (bkl): weak tosca.

The results of TLC analyse of methanol extract with the mobile phase of n-hexane: aethyl acetate (7: 3) using a 50% sulfuric acid in methanol as visualization gave 20 spots namely green, dark green, weak green, yellow, weak yellow and yellowish green. The appearance of 5% iron (III) chloride as visualization gave ten spots, namely blackish green, blue, and green indicating polyphenol compounds. The appearance of Liebermann-Burchard as visualization gave three spots (tosca and weak tosca) which showed that the presence of steroid compounds and the Dragendroff as visualization appearance gave two spots, namely brown colour indicating the presence of alkaloid compounds [9].

Table 3: Result of TLC Chromatogram of Methanol Extract of Lidah Mertua Leaves

Mobile phase: *chloroform*-aethyl acetate (3: 7)	Visual	H_2SO_4 50% in methanol	$FeCl_3$ 5%	Lieberman-Bourchard	Dragendroff
Methanol extract	-	0.91 (ht) 0.87 (k) 0.66 (k) 0.26 (h) 0.22 (h) 0.17 (hk) 0.13 (h) 0.05 (c)	0.92 (hk) 0.23 (hk)	0.95 (h) 0.83 (h) 0.18 (h) 0.16 (h)	0.2 (jk)

Note: (ht): dark green; (k): yellow; (h): green; (hk): blackish green; (jk): brownish orange; (c): brown.

The results of TLC chromatogram of methanol extract with the mobile phase of chloroform: aethyl acetate (3: 7) using a 50% sulfuric acid in methanol as visualization gave eight spots namely green, dark green, yellow and brown. The appearance of 5% iron (III) chloride as visualization gave two spot, namely blackish green indicating polyphenol compounds. The appearance of Liebermann-Burchard as visualization gave four spots, namely green, indicating that the presence of steroid compounds and Dragendroff's as visualization gave one stain, namely brownish orange colour indicating the presence of alkaloid compounds [9].

Antibacterial Activity of Lidah Mertua Leaves Extract (Sansevieria trifasciata Prain) Against Escherichia coli

Escherichia coli culture used in this study was pure culture from the Laboratory of Microbiology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, which was then cultured on Nutrient Agar (NA) media at 37°C for 24 hours. The concentration results of n-hexane, ethyl acetate and methanol extract of the Lidah Mertua leaves were 300 mg/mL; 200 mg/mL; 100 mg/mL; 50 mg/mL; 25 mg/mL; 12.5 mg/mL; 6.25 mg/mL; 3.125 mg/mL.

Antibacterial activity results showed that aethyl acetate extract of Lidah Mertua leaves could inhibit the growth of *Escherichia coli* bacteria at a concentration of 300 mg/ml (Figure1).

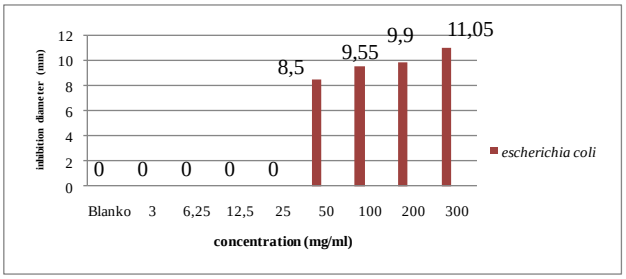

Figure 1: Graph of Test Results of Ethylacetate Extract as Antibacterial Activity on Escherichia coli (Note: Inhibited diametre has not been reduced by a metal propeller diameter of 8 mm)

The Minimum Inhibitory Concentration (MIC) of ethyl acetate extract of lidah mertua leaves was 50 mg/ml with an inhibitory diameter of 8.50 mm.

Based on the graph in Figure 2, the results of antibacterial test activity showed that the Methanol extract of the *Lidah Mertua* leaves could inhibit the growth of *Escherichia coli* bacteria at a concentration of 300 mg/mL, 200 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL and 12.5 mg/mL with inhibition power of 11.29 mm, 10.43 mm, 10.2 mm, 9.83 mm, 9.15 mm and 8.46 mm. The minimum inhibitory concentration (MIC) is found at the concentration of 12.5 mg/mL with an inhibitory diameter of 8.46 mm.

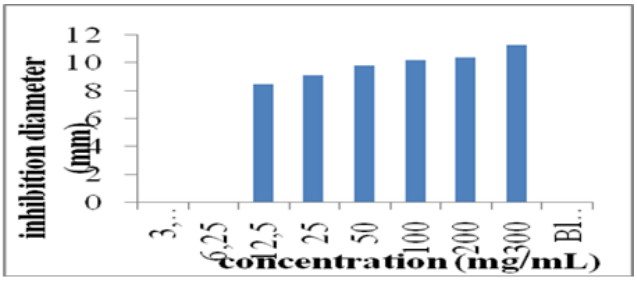

Figure 2: Graph of Testing Results of Antibacterial Activity of Methanol Extract against Escherichia coli (Note: Inhibited diametre has not been reduced by a metal propeller diameter of 8 mm)

Results of Antibacterial Activity Test of Lidah Mertua Leaves (Sansevieria trifasciata Prain) Extract on Staphylococcus aureus

The culture of *Staphylococcus aureus* used in this study was pure culture derived from the Laboratory of Microbiology, Faculty of Pharmacy, University of North Sumatra, Medan which was then cultured on Nutrient Agar (NA) media at 37°C for 24 hours.

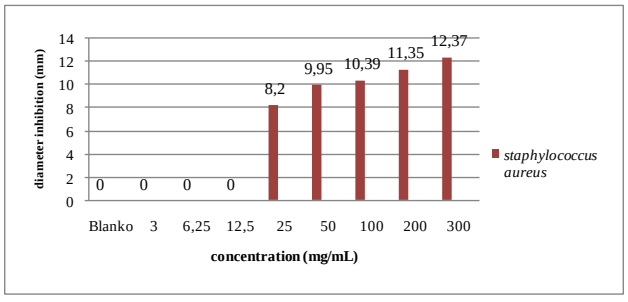

Figure 3: Graph of Antibacterial Activity of Ethylacetate Extract against Staphylococcus aureus (Note: Diametre has not been reduced by a metal propeller diametre of 8 mm)

From the graph (Figure 3), it can be seen that antibacterial activity shows that aethyl acetate extract of the *Lidah Mertua* leaves can inhibit the growth of *Staphylococcus aureus* bacteria at a concentration of 300 mg/ml. The Minimum Inhibitory Concentration (MIC) of ethyl acetate extract of *lidah mertua* leaves was 50 mg/ml. The results of the methanol extract of the *Lidah Mertua* leaves in concentration of 300 mg/mL; 200 mg/mL; 100 mg/mL; 50 mg/mL; 25 mg/mL; 12.5 mg/mL and 6.25 mg/mL can be seen in the graph 4.

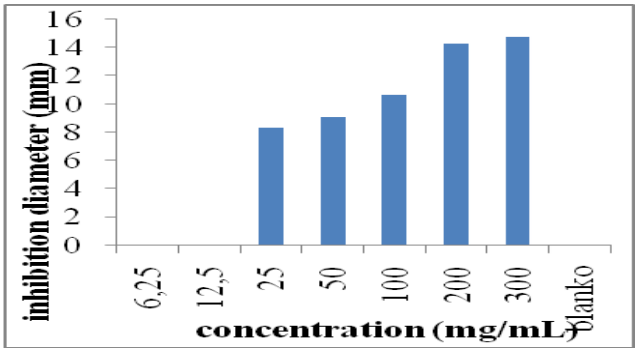

Figure 4: Graph of the results of Antibacterial Activity of Methanol Extract against Staphylococcus aureus (Note: Diametre has not been reduced by a metal propeller diametre of 8 mm)

Based on the graph above, the results of antibacterial activity showed that the methanol extract of *lidah mertua* leaves could inhibit the growth of *Staphylococcus aureus* bacteria at a concentration of 300 mg/mL, 200 mg/mL, 100 mg/mL, 50 mg/mL and 25 mg/mL with each inhibitory -14.75 mm; 14.27 mm; 10.64 mm; 9.09 mm and 8.325 mm. The minimum inhibitory concentration (MIC) is found at a concentration of 25 mg/ml with a diameter of 8.325 mm inhibitory.

Discussion

In testing the antibacterial activity with n-hexane extract, it cannot inhibit the growth of *Escherichia coli* bacteria. Fardiaz, 1992 [10] suggested that the speed and efficiency of bacterial damage by antibacterial compounds is influenced by temperature, pH, time, concentration and the presence of other organic components. Other organic compounds can reduce the activity of antibacterial substances by activating and disrupting contact between antibacterial substances and bacterial cells, to protect bacteria from antibacterial substances. This is because medicinal plants still contain organic material other than antibacterial, so it requires further purification stages to obtain pure extracts that only contain antibacterial compounds. Besides, the extract used is a crude extract, which contains various active compounds which each have different effects in inhibiting bacterial growth. This does not mean causing synergism when various active compounds are mixed but it is likely to cause the optical power of the active compound to work [11]. Based on the results obtained that the *n*-hexane extract of the *lidah mertua* (*Sansevieria trifasciata* Prain.) could not inhibit the growth of *Staphylococcus aureus* bacteria. The

inhibitory zone that is not formed is thought to be because the antibacterial material cannot pass through the bacterial cell wall so that it does not affect the growth of the bacteria. In addition *Staphylococcus aureus* bacteria have a thick cell wall and remove a compound its character damages the antibacterial activity of the compound is the most common beta-lactamase produced by the *Staphylococcus aureus* bacteria so that the bioactive compounds contained in the *leaves* extract of the *lidah mertua* (*Sansevieria trifasciata* Prain.) are unable to perform the inhibitory mechanism. Each antimicrobial material has an optimum inhibitory time varying because it is assumed that the content and working system of the antibacterial compounds contained in antimicrobial materials differ.

Based on the Indonesian Pharmacopoeia, 1995 [12], the requirements of the inhibitory area are effective when producing an area of resistance with a diameter of approximately 14 mm. According to Fatima Wali and Wiyono, 2012 [13], the strength criteria for antibacterial inhibition are as follows: 5 mm inhibition zone diameter or less is categorised as weak, 5-10 mm inhibition zone is categorized as moderate, 10-20 mm inhibition zone is categorized as strong and 20 mm zone or more strongly categorized. Based on these criteria, the antibacterial power of ethyl acetate extract on *Staphylococcus aureus* and *Escherichia coli* bacteria was in the strong category starting at 300 mg/ml, while the weak inhibition zone of ethyl acetate extract from the *Lidah Mertua* leaves on *Staphylococcus aureus* at a concentration of 12.5 mg/ml and the weak inhibition zone of ethyl acetate extract from the *Lidah Mertua* leaves in *Escherichia coli* bacteria was 50 mg/ml. Based on the results of the TLC the ethyl acetate extract of the *Lidah Mertua* leaves showed the presence of alkaloids, polyphenols, and steroids.

Based on the results of the study, it was found that the leaves extract of the lidah mertua (*Sansevieria trifasciata* Prain.) had antibacterial ability against *Escherichia coli* and *Staphylococcus aureus* bacteria. This is due to the presence of active substances contained in the *Lidah Mertua* plant. Active substances contain in the extract of Lidah Mertua leaves which may inhibit the growth of bacteria, namely saponins, phenols, and flavonoids. Saponin is a type of glycoside that is found in plants. Saponins have characteristics in the form of foam [14]. Phenol is a compound with an -OH group which is attached directly to an aromatic ring. Phenol compounds are abundant in nature and are intermediates for industries for various kinds of products such as adhesives and antiseptics while flavonoid compounds are a group of the largest phenol compounds found in nature. The above compounds have antiseptic, anti-inflammatory and anti-cancer effects [15].

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