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Research Article

Test Of Antibacterial Activity of Ethanol Extract of Maman Lanang Plant (*Cleome rutidospermae dc*) Against Gram Positive and Negative Bacteria (*Staphylococcus aureus and Eschericihia coli*)

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Abstract

The Maman Lanang plant (*Cleome rutospermae dc)* is a weed plant that is often found in rice fields or grows on community cultivated plants which are usually used as food for people on the outskirts. This research aims to determine the effect of the extract concentration of the Maman Lanang Plant (Cleome *rutospermae dc)* on the inhibition zone for the growth of *Staphylococcus aureus* and *Escherichia coli bacteria,* as well as to find out how much the concentration of the extract from the Maman Lanang Plant (*Cleome rutospermae dc*) is close to the inhibitory zone value of the positive control of tetracycline in inhibiting the growth of *Staphylococcus aureus* and *Escherichia coli bacteria*. This research is a laboratory experimental study using a Completely Randomized Design (CRD) using the hole/cup diffusion method with four varying concentrations, namely 10%, 30%, 60% and 90%, and the positive controls used are tetracycline and distilled water as a negative control. The main ingredient used in this research was the Maman Lanang plant (*Cleome rutospermae dc*) which was extracted using the maceration method for three days with 96% ethanol solvent, after that the extract obtained was then subjected to an evaporator to obtain the crude extract then carried out a phytochemical test and after that The antibacterial activity of the crude extract from the Maman Lanang Plant (*Cleome rutospermae dc)* was tested against the bacteria *Staphylococcus aureus* and *Escherichia coli* . Then the data obtained was analyzed using the antibacterial ANOVA (*Analysis of variance*) method. The results of this study show that phytochemical test analysis shows the presence of flavonoid compounds in extracts from the Maman Lanang Plant (*Cleome rutospermae dc*). Furthermore, the analysis showed that variations in concentration of the Maman Lanang Plant (*Cleome rutospermae dc*) had an influence on the antibacterial activity *of Staphylococcus aureus* and *Escherichia coli.* Where the higher the concentration of the extract, the larger the inhibition zone will be formed. The 90% concentration of the extract showed the largest inhibition zone with a diameter of 23.3 mm.

Keywords: Maman Lanang plant, antibacterial, *Staphylococcus aureus* and *Escherichia coli.*

1. Introduction

Indonesia is a country with various surface shapes and regions with different climatic conditions. Indonesia also has various kinds of high biodiversity. The large amount of biodiversity means that Indonesia has various types of medicinal plants (Novianti, 2017).

The use of extracts from natural ingredients has long been used empirically for treatment. These extracts are used because they contain bioactive compounds that can provide pharmacological effects. The isolate from the extract was tested both in vitro and in vivo to determine the effects and bioavailability in the body scientifically. (Ramadon, 2016).

Traditional medicine is an ingredient or concoction of ingredients in the form of plant materials, animal materials, mineral materials, extract preparations (galenic) or mixtures of these materials which have been used for generations for treatment, and can be applied in accordance with the norms applicable in society (BPOM, 2014).

Maman Lanang *(Cleome rutidospermae DC)* is a plant that is commonly cultivated because of its presence in the wild. Some people use it as a herbal medicine to treat swelling, pain or redness in the eyes, because the identification results of fraction I (FI) show the presence of steroid, flavonoid and phenolic compounds. (Wahyuni, 2018).

Diarrhea is a condition where there is a lot of fluid in the water (loose stools) and is a symptom of certain diseases or other disorders. One of the causes of diarrhea is bacteria, which occurs frequently, but is starting to decrease due to the increasing level of hygiene in society. Germs in certain circumstances become *invasive* and invade the mucosa, where they multiply while forming toxins. This enterotoxin can be absorbed into the blood and cause severe symptoms, such as high fever, headaches and convulsions. Apart from that, damaged intestinal mucosa results in bloody and slimy diarrhea. (Tjay, 2015).

Escherichia coli is a *gram*- negative enteric bacterium (*Entero bactericeae*), a normal flora bacterium found in the human large intestine. These bacteria are pathogenic if they are outside the intestine, namely the normal location where they are and other places where these bacteria rarely live. *Escherichia coli* often causes infections in the urinary tract, bile ducts and other places in the abdominal cavity. *Escherichia coli* is also a cause of diarrhea and urinary tract infections. (Nova, 2017).

Staphylococcus aureus is a gram-positive type of bacteria which is estimated to be found in 20-75% of the upper respiratory tract, face, hands, hair and vagina. This bacterial infection can cause disease with characteristic signs, namely inflammation, necrosis, acne, hair follicle infection, and abscess formation. Among the organs that are often attacked by *Staphylococcus aureus* bacteria is skin that is injured and it can spread to other people who also have injuries. Lesions caused by *Staphylococcus aureus bacteria* can be seen in abscess lesions or acne. Bacteria invade and multiply in hair follicles causing cell death or necrosis in local tissue. This is followed by a buildup of inflammatory cells in the cavity. So, there is an accumulation of pus in the cavity. This accumulation of pus causes pressure on the surrounding tissue and walls are formed by healthy cells, thus forming an abscess. These bacteria can also spread to other parts of the body via lymph vessels and blood vessels so that there is also inflammation of the veins and thrombosis (Razak, 2013).

Empirically, the Maman Lanang plant (*Cleome rutospermae dc*) has several benefits that are used by the community and has the potential to cure several diseases. Maman lanang leaves are known to be able to treat anti-diarrhea, eye medicine and anti-inflammatory diseases, so researchers are interested in researching maman lanang plants (Wahyuni, 2018).

Based on the results of research conducted by previous researchers (Chakrabity, et al. 2010), regarding comparative studies of antioxidant activities between ethanolic extracts and aqueous extracts *of cleome rutidospermae*. And the results of this research explain

that the maman lanang plant has been used empirically as a treatment for paralysis. However, no one has carried out continuous testing, therefore researchers are interested in testing the maman lanang plant as an antibacterial extract.

2. Materials and Methods

2.1 Materials

Volume pipette, suction ball, analytical balance, *hot plate stirrer,* filter paper, tongs, blender, 40 mesh sieve, scales, jar container, knife, black cloth, porcelain cup, oven, *rotary evaporator,* filter paper, glass funnel, aluminum foil, 500 ml *beacker glass* and 250 ml, test tube rack, 500 ml Erlenmeyer, incubator, petri dish, micropipette, aluminum foil paper, disc paper, test tube cover, caliper, label paper. Autoclave, ruler, petri dish, glass stir bar, 1000 ml measuring cup, measuring flask, tweezers, Bunsen lamp and incubator, gauze, round tube, test tube, cotton and, blue tip. Maman Lanang plant (*Cleome rutidospermae dc*), 96% ethanol, and distilled water. tetracycline solution as positive control, distilled water as negative control, NA (Nutrient Agar), *Escherichia coli* and *Staphylococcus aureus* bacteria. Hydrochloric acid, bouchardate, potassium dichromate, ethanol, sulfuric acid.

2.2 Methods

Plant Determination

Plant determination was carried out at the Biology Laboratory-FMIFA, University of Mataram. The aim of plant determination is to find out the correct identity of the Maman Lanang Plant (*cleome rutidospermae dc*) so as to avoid errors in sampling.

Sampling

Collect maman lanang plants from young to not too old, where the plants are green, fresh, and not damaged. Collection was carried out by picking one by one, 2.8 kilo grams were taken in Bilebante Village, Central Lombok.

Sample Extraction

A total of 150 grams of powder was put into a glass jar, then 96% ethanol was added using a glass funnel, then maceration was carried out. Maceration using 1 liter of 96% ethanol solvent, soaked for 3 days with occasional stirring. The maceration results are then filtered with filter paper to produce a filtrate and residue. The maceration results are then filtered using 4 (four layers) layered filter paper so that there are no leaks during filtration and concentrated using a *rotary evaporator.* After that, the pure extract obtained is evaporated in an oven at a temperature of 40 0^{C} until *the* alcohol evaporates and a thick extract is formed, then calculate the resulting yield (Sapara *et al,* 2016).

Phytochemical Screening

Testing for flavonoid compounds in the Maman Lanang plant (*Cleome rutidospermae dc*) is by adding 1 ml of the Maman Lanang plant sample, then adding Mg powder and 3 drops of concentrated HCl, if it changes to orange or red, it indicates the presence of flavonoid compounds.

Bacterial Cultivation

Preparation of Bacterial Stock *Escherichia coli* and *Staphylococcus aureus* were obtained at the Biology Laboratory, FMIPA, Mataram University. Making Bacterial Cultures. The test bacteria are taken using a sterile tube needle, then embedded on NA agar media by streaking. Next, it was incubated in an incubator at 37 ^{0}C for 24 hours (Eva, 2017).

Preparation of Bacterial Suspensions

The test bacteria that have been inoculated with a sterile tube needle are then suspended into a test tube containing 3 milliliters of 0.9% sodium chloride (NaCl) solution until the *Mc standard is obtained. Farland.*

Making Mc. Farland

9.5 ml of 1% sulfuric acid solution was put into a test tube. Add 0.5 ml of 1% barium chloride solution. shaken until homogeneous (Novita *et al,* 2017).

Nutrient agar (NA) media

A total of 6 grams of NA was dissolved in 300 milli liters of distilled water then heated and stirred using a magnetic stirrer hot plate until homogeneous. Next, the media was sterilized using an autoclave at a temperature of 121˚C, a pressure of 2 atm and for 15 minutes. This medium will be used in antibacterial testing. (Yati, Y, 2017).

Bacterial Activity Test

NA media was poured into a petri dish and mixed with 0.1ml each of *Escerichia coli* and *Staphylococcus aureus bacterial solutions.* After that, it is homogenized and left to solidify. Then, the solidified agar medium is smeared with each bacterium and a well is made, then the Maman Lanang extract solution is added. The petri dish was incubated at 37°C for 24 hours until an inhibitory area appeared. The inhibition zone was measured with a caliper to determine antibacterial activity (Jannah, 2017).

3. Results and Discussion

3.1 Results

Extraction (Maceration)

Maceration is a simple method of extraction. Maceration is done by soaking simplicia powder in filter fluid. In the maceration process on the maman lanang plant (*Cleome rutidospermae dc*) by taking a sample of 150 grams of dried simplicia then adding 1 liter of 96% ethanol solvent, the solution is poured into a jar containing dry simplicia then stirred until mixed (submerged) and left to sit for 3 days, stirring occasionally during the soaking process.

Phytochemical Screening

Phytochemical Test Results

Table 2. Phytochemical test results

Phytochemical screening	Reagent	Literature	Results	Information
Flavonoids	- Mg powder - HCL	- Orange -Brownish red	Brownish red	Positive (+)

Ethanol Free Test Results

Table 3. Ethanol-free test results

Test	Material	Literature	Results	Information
Ethanol free	- Sulfuric acid - Potassium dichromate	If it contains ethanol, it will form a blue color	Brownish red	Negative (-)

Medium Creation

Nutrient Agar Media

Staphylococcus aureus and *Escherichia coli* bacteria is by weighing 6 grams of NA (Nutrient Agar) dissolved in 300 ml of distilled water then heated and stirred using a magnetic stirrer until it is homogeneous and looks clear. Then the media was sterilized using an autoclave at a temperature of 121˚C, a pressure of 2 atm and for 15 minutes. This medium will be used in antibacterial testing. (Yati Y, 2017).

Liquid Media (NaCl)

Weigh out 0.9 grams of NaCl. Put it in an enlemeyer, then dissolve it with 100 ml of distilled water, stir until smooth then pour 9 ml of NaCl solution into a test tube, cover with aluminum foil, rinse and sterilize using an autoclave.

Diffusion Method (Well Method)

The well method is carried out by making perpendicular holes in solid agar that has been inoculated with the test bacteria. In the plate that has been inoculated with the test bacteria, a hole is made which is then filled with the test antimicrobial substance. Then each hole is filled with the test substance. After incubation at a temperature and time appropriate to the test microbes, observations were made to see whether or not there was an obstacle zone around the hole (Siti N, 2020).

Results of Observation of Antibacterial Activity

a. *Staphilococcous Aureus* bacteria

Test the antibacterial activity of Maman Lanang plants (*Cleome rutidospermae dc) against Staphilococcous Aureus* bacteria with various concentrations, namely 10%, 30%, 60%, and 90% which were added to agar media that had been treated with the well method. For negative control treatment with distilled water and positive control with tetracycline antibiotics.

Table 4. Average value of *Staphilococcous Aureus bacteria*

Treatment	Average clear zone diameter (mm)
Concentration 10%	12
Concentration 30%	16
Concentration 60%	17
Concentration 90%	22
Control (+) Tetracycline	40
Control (-) Aquades	0

By looking at the concentration from the table above, it can be concluded that the concentration is 10%, 30%, 60% and 90% after measuring the inhibition zone so that the results and averages can be drawn after the measurement.

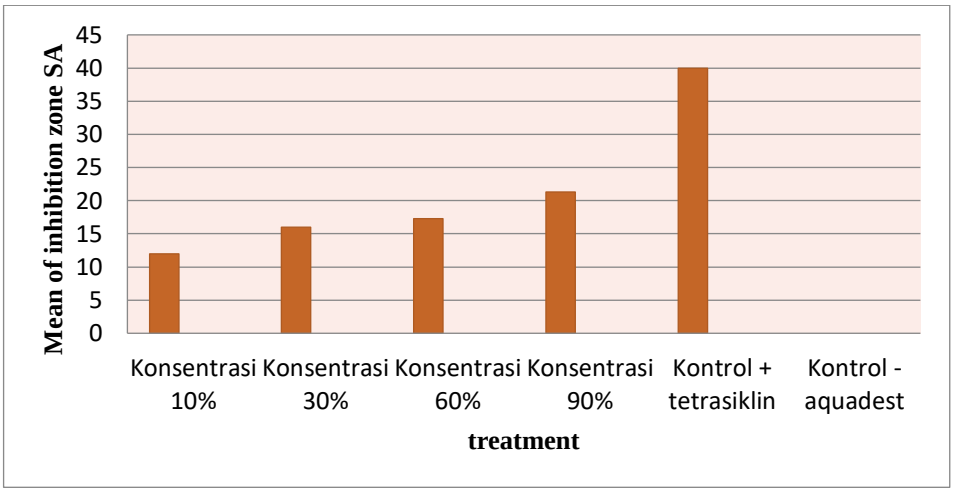

Figure 1. ***Mean*** **of the inhibition zone** ***of Staphilococcous Aureus***

With 3 repetitions for each treatment, 3 categories of differences were obtained where each treatment had a significant difference, namely 0.077 - 1.000. Control (-) has an inhibition zone diameter of 0.000. A 10% concentration has an inhibition zone diameter of 12,000. A 30% concentration has an inhibition zone diameter of 16,000. A concentration of 60% has an inhibition zone diameter of 17,333. A concentration of 90% has an inhibition zone diameter of 21,333. Control (+) Tetracycline has an inhibition zone diameter of 40,000. The higher the concentration of Maman Lanang (*Cleome Rutidospermae DC*) plant extract, the longer the diameter of the inhibition zone against *Staphulococcous Aureus*.

b. *Escherichia Coli* bacteria

Test the antibacterial activity of Maman Lanang plants (*Cleome rutidospermae dc) against Eschericihia Coli* bacteria with various concentrations, namely 10%, 30%, 60%, and 90% which were added to agar media that had been treated with the well method. For negative control treatment with distilled water and positive control with tetracycline antibiotics.

Table 5. Average value of *Eschericihia coli bacteria*

Treatment	Average clear zone diameter (mm)
Concentration 10%	11.6
Concentration 30%	15.6
Concentration 60%	17.6
Concentration 90%	23.3
Control (+) Tetracycline	22
Control (-) Aquades	0

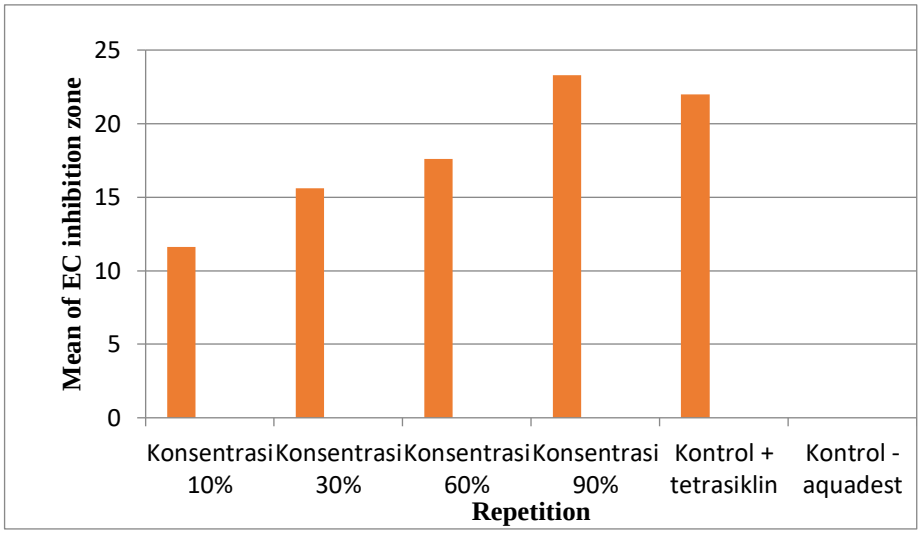

FIgure 2. *Mean of Eschericihia coli* inhibition zone

With 3 repetitions for each treatment, 3 categories of differences were obtained where each treatment had a significant difference, namely 0.150 - 1.000. Control (-) has an inhibition zone diameter of 0.000. A 10% concentration has an inhibitory zone diameter of 11.667. A concentration of 30% has an inhibitory zone diameter of 15.667. A concentration of 60% has an inhibition zone diameter of 17,667. A concentration of 90% has an inhibitory zone diameter of 23,333. Control (+) Tetracycline has an inhibition zone diameter of 22,000. The higher the concentration of Maman Lanang (*Cleome Rutidospermae DC*) plant extract, the longer the diameter of the inhibition zone against *Eschericihia Coli*.

Conclusion

1. The ethanol extract of the Maman Lanang plant (*Cleome rutospermae dc*) has an antibacterial effect against *Staphylococcus aureus* and *Escherichia coli bacteria.* The higher the concentration value, the higher the zone of inhibition or activity.

2. The ethanol extract of the Maman Lanang plant (*Cleome rutospermae dc*) which has a greater inhibition zone than tetracycline is at a concentration of 90% with an inhibition zone diameter value of 23.3 mm which is included in the very strong category.

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