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Article

Sensitivity Test of Herbal Extracts of Jarem Grass (*Grona triflora*), Meniran (*Phyllanthus niruri*), and Patikan Kebo (*Euphorbia hirta*) Against Terrestrial Bacteria

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Abstract: Sensitivity is the ability of a medicinal substance to kill or inhibit the growth of microbes. Meanwhile, intermediate is a condition where there is a shift from a sensitive state to a completely resistant state. Method research was experimental and several procedural processes were sterilization, making of MHA media, bacterial cultivation, and extract preparation. Result showed that the highest diameter of the inhibition zone for the extract was found in the *Grona triflora* extract in group 1, which was 35 mm and followed by *Phyllanthus niruri* extract in group 6 which was 29 mm. Furthermore, the inhibition zone diameter of positive control, ciprofloxacin and tetracyclines, were 40 mm. The active ingredients of *Grona triflora* are flavonoids, alkoloids, and phenolics and the components of meniran that are flavonoid and tannin compounds are estimated as the inhibition factor that cause the inhibition zone in the media. Finally, the inhibitory zone of grass jarem herb extract (*Grona triflora*) (EGT) and the inhibitory zone for extract of *Phyllantus niruri* (EPN) are called to have the similar level of sensitivity when compared with several types of antibiotics ciproploxacin and tetracycline which have the sensitive level ≥ 21 mm.

Keywords: Sensitivity, Plant Extract, Inhibition zone.

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1. Introduction

Sensitivity is the ability of a medicinal substance to kill or inhibit the growth of microbes. Meanwhile, intermediate is a condition where there is a shift from a sensitive state to a completely resistant state. Then the resistance state is the resistance of microorganisms to an antibiotic.

Antibiotic sensitivity testing is a method for determining the level of susceptibility of bacteria to antibacterial substances (antibiotics) and to determine pure compounds that have antibacterial activity. The examination can be carried out in two ways: firstly, by sensitivity testing using the Kirby Bauer diffusion test method and secondly by dilution testing [1].

The principle of this method is to inhibit the growth of microorganisms. The inhibition zone is visible as a clear area around the paper disc containing antibacterial substances. The diameter of the inhibition zone for bacterial growth indicates the sensitivity of the bacteria to antibacterial substances. Furthermore, it is said that the wider the diameter of the inhibition zone formed, the greater the sensitivity of a plant extract [1].

In this study, the inhibitory zones for extracts of jarem grass (*Grona triflora*), patikan kebo (*Euphorbia hirta*), and meniran (*Phyllanthus niruri*) were measured because ethnomedicinally they are often used by people around Lombok, West Nusa Tenggara to treat wounds.

The inhibition zone is related to bacterial cells that do not grow. Bacterial cells vary greatly in length; the cells of some species can be 100 times longer than the cells of other species. The unit of measurement for bacteria is the micrometer (μm), which is equivalent to 1/1000 mm or 10^{-3}mm. The bacteria most commonly studied in microbiology research measure approximately 1.0 x 2.0-5.0 μm. Average-sized rods such as typhoid and dysentery bacteria have a width of 0.5 to 1 μm and a length of 2 to 3 μm. The cells of some bacterial species are very long; their length can exceed 100 μm and their diameter ranges from 0.1 to 0.2 μm. A group of bacteria known as microplasma, are typically very small in size - so small that they are almost invisible under a light microscope. They are also pleomorphic; that is, their morphology is very diverse. Their sizes range from 0.1 to 0.3 μm [2].

The validity of the antimicrobial susceptibility test (AST) depends on the rigorous standardization of each test characteristic, but not limited to the composition of the medium used. This is necessary to obtain effective results for the correct evaluation of the patient's treatment regimen [3].

In parasitology microbiology research, there are several steps that need to be taken to ensure sterile conditions, making MHA (Mueller Hinton Agar) media, observing colonies, counting the number of bacterial cells, and testing sensitivity to antibacterial. Sterilization aims to kill or destroy all microbial organisms in tools, materials or work spaces to prevent contamination. Making MHA media is an important step in bacterial culture, while observing colonies and counting the number of bacterial cells is used to obtain information about the bacteria present. Sensitivity tests are used to determine the sensitivity or resistance of bacteria to an antibiotic [3].

To obtain the pure bacteria, researcher should do the sterilization process. Sterilization can be carried out mechanically (for example by radiation), chemically (with disinfectants), or physically (for example by heating, UV rays, X rays). The sterilization method used depends on the type and nature of the material being sterilized. One way of sterilization is to use an autoclave. An autoclave is a pressure-resistant vessel equipped with a manometer, thermometer and safety valve. It should be noted that sterilized materials and tools are materials and tools that are not damaged by heat and high pressure. Sterilization using an autoclave is the best sterilization method because high pressure hot water steam increases the penetration of water vapor into microbial cells so that plasma proteins experience coagulation. Plasma protein coagulation is what accelerates the process of microbial death [4].

2. Materials and Methods

This is a true experimental research. The research design used was post-test only with control group design. Inhibition zone of ciprofloxacin and tetracyclines are as the positive control, meanwhile inhibition of plant extract is as treatment control. Plant extracts consist of *Grona trifloral* extract and *Phyllanthus niruri* extract as the sample research. This research was carried out at the Pharmacy Laboratory, Faculty of Health Sciences, Nahdlatul Wathan University, Mataram from 6-8 July 2024. The procedures to collect data are is below.

Sterilization

The steps taken in sterilization means water is put into the bottom of the autoclave until flooding the bottom of the autoclave. Next, tools or the material to be sterilized is put inside autoclave. Then install the autoclave lid and screw it on tightened and the steam release control valve opened. Finally, the autoclave heater is installed (can be electric or

fire). When water vapor comes out (makes a sound hissing), the steam outlet valve is closed to the pressure in the autoclave increases to ± 2 atm and the temperature 121^{0}C. When the desired temperature and pressure has been reached, reduce heating.

Making of MHA Media

The steps for making MHA media are as follows: carefully weighed MHA as much as 3.8 grams. Next is the MHA Put it in a beaker glass, then dissolve it with distilled water to 100 ml. Then dissolve the MHA until homogeneous, then put it in Erlenmeyer and heat. After the solution boil, place in a petri dish, and let sit until cold. Sterilize using an autoclave pressure 2 atm and temperature 121^{0}C for 30 minutes.

Bacterial cultivation

The steps for cultivating bacteria are as follows: prepare a sample containing terrestrial bacteria as much as 1 gram. Next is the sample is put into the first test tube 10^{-1} then dissolve with 9 ml of distilled water, stir until homogeneous. Take 1 ml from tube 10^{-1} with Pipette the drop then transfers it into the tube 10^{-2} then add 9 ml of distilled water. Transfer until tube 10^{-7} in the same way.

Preparation of plant extracts

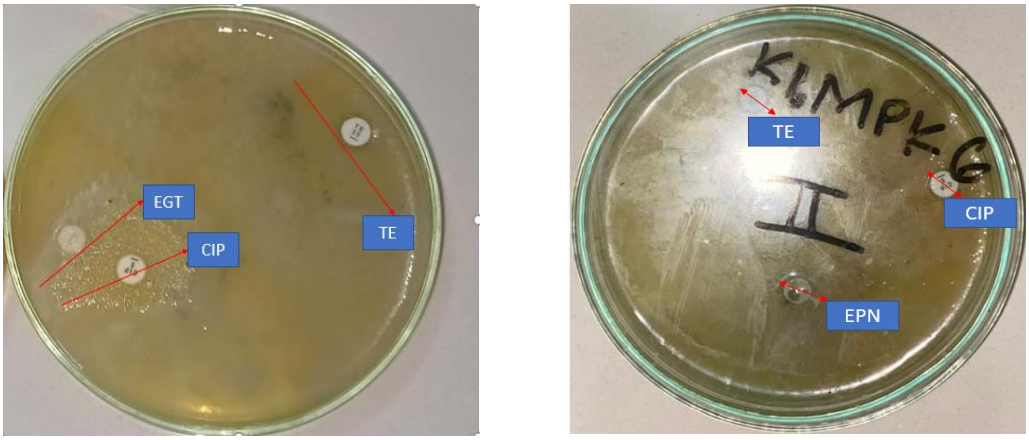
Plant extracts are prepared by first cleaning the plant, then drying the plant briefly to remove the water content in the plant. Next, the plants are crushed and then diluted with distilled water with a concentration of 100%. Put a sterilized paper disk into the 100% plant extract. Finally, the paper disk is planted into a medium that has been planted with terrestrial bacteria.

3. Results and Discussion

In this research, terrestrial bacteria were used. The extracts tested were 3 extracts consisting of *Grona triflora* extract, *Euphorbia hirta* extract, and *Phyllantus niruri* extract. Meanwhile, the antibiotics used as positive controls were ciprofloxacin and tetracyclines.

3.1 Sensitivity test on MHA media

Figure 1 showed that there was an inhibitory zone in the MHA media which was characterized by no growth of bacteria in the area of the antibiotic and plant extract tested.



(**a**) (**b**)

Figure 1. Figure of inhibition zone. (**a**) Inhibition zone of EGT = extract of *Grona triflora*, CIP= Ciprofloxacin, TE= Tetracyclines; (**b**) Inhibtion zone of EPN = Extract of *Phyllanthus niruri*, CIP= Ciprofloxacin, TE= Tetracyclines.

3.2 Inhibition zone measurement

After incubation, the sizes of the zones were measured and interpreted. The size of the zone of inhibition depends on antibiotic and extract for the strain tested and on the size of the antibiotic and extract molecule (Table 1).

Table 1. Diameter of inhibition zone.

Group	Ciprofloxacin (mm)	Tetracyclines (mm)	Plant extracts (mm)
1	30	40	EGT : 35
2	40	20	EGT : 0
3	45	30	EEH : 0
4	40	5	EGT : 0
5	30	0	0
6	20	24	EPN : 29

* EGT = Extract of *Grona trifloral,* EEH = Extract of *Euphorbia hirta,* EPN = Extract of *Phyllanthus niruri*

Table 1 showed that the highest diameter of the inhibition zone for the extract was found in the *Grona triflora* extract in group 1, which was 35 mm and followed by *Phyllanthus niruri* extract in group 6 which was 29 mm. Furthermore, the inhibition zone diameter of the positive control in Ciprofloxacin and Tetracyclines were 40 mm.

In the first cup, the results were obtained that the inhibitory zone of grass jarem herb extract (*Grona triflora*) (EGT) on MHA media is 35 mm (Figure 2.a), while on the second plate the diameter of the inhibitory zone for extract of *Phyllantus niruri* (EPN) was 29 mm (Figure 2.b). Furthermore, diameter of inhibition zone for *Euphorbia hirta* extract was not shown because of the 0 mm diameter. Data for EGT and EPN are called to have the similar level of sensitivity when compared with several types of antibiotics ciprofloxacin and tetracycline which have the sensitive level ≥ 21 mm [5].

The results of research from other researchers regarding *Grona triflora* explain that the phytochemical compounds of medicinal plants such as *G. triflora* and *H. indicum* have similarities to drugs and bind actively with candidapepsin2, so this illustrates that these two plants can be used to make better candidiasis therapy. The research explains that indicine-N-oxide is an active compound that plays an important antitumor role. The current study can illustrate that indicine-N-oxide also has another role as a better antifungal against candidapepsin2 when compared to all other phytochemical compounds and standard drugs used for research. Therefore, the presence of this active compound may play an important role as a traditional medicine to fight infections for several years. With O–H and H–O bonds with bond lengths of 2.0 and 1.9 Å respectively, the standard compound also effectively binds to the target. From the docking results it is clear that the components contained in *Grona triflora* and *Heliotropium indicum* provide greater efficiency than standard drugs against *Candida albicans* [6].

The active compounds of *Grona triflora* are flavonoids, alkoloids, ethanol, indole-3-acetic acid, trigoneline, choline, hypaphorine, saponins, terpenoids, anthocyanins, tyrumin, steroids, phenolics, amino acids. In Indonesia, Malaysia, the Philippines, Thailand, China, India and Sri Lanka, *Grona triflora* infusion is usually consumed to treat various diseases such as diarrhea and dysentery, as a mouthwash and expectorant. The crushed plant or folium poultice is applied topically to wounds, boils, and general skin problems. This is done because *G. trifloral* has antiseptic properties [7].

The components of *Phyllanthus niruri* that are immunomodulatory are flavonoid compounds which are able to improve the immune system so that it can ward off attacks by viruses, bacteria or other microbes [8]. *P. niruri* can be used as an antibacterial because it contains several compounds that act as antibacterial including flavonoids, tannins, phenols and alkaloids [9].

Flavonoids are known to be antibacterial compounds against many pathogenic microorganisms. With the increasing prevalence of untreatable infections caused by antibiotic-resistant bacteria, flavonoids are attracting the attention of researchers because of their potential to replace antibiotics. The presence of hydroxyl at certain locations in the aromatic ring of flavonoids can increase the therapeutic activity of these compounds. However, the methylation process of active hydroxyl groups can generally reduce flavonoid activity. In addition, the lipophilicity of the A ring plays an important role in chalcone activity. Hydrophobic components such as prenyl groups, alkylamino chains, alkyl chains, and heterocyclic groups containing nitrogen or oxygen can increase the activity of all flavonoids. The antibacterial mechanisms of flavonoids are as follows: nucleic acid synthesis inhibition, cytoplasmic membrane function inhibition, energy metabolism inhibition, inhibition of attachment and biofilm formation, inhibition of porins on cell membranes, changes in membrane permeability, and attenuation of pathogenicity [10]. The mechanism of action of flavonoids as antimicrobials can be divided into 3 types. Firstly, by inhibiting nucleic acid synthesis, secondly by inhibiting membrane function cells and lastly by inhibiting energy metabolism [11]. Antibacterial mechanism flavonoids which inhibit nucleic acid synthesis are rings A and B plays an important role in the process of interchelation or hydrogen bonding by accumulating nucleic acid bases that inhibit formation DNA and RNA. The location of the hydroxyl group in the 2',4' or 2',6' position is hydroxylated on ring B and 5,7 dihydroxylation on ring A plays an important role on the antibacterial activity of flavonoids. Flavonoids cause this damage to the permeability of bacterial cell walls, microsomes and lysosomes as a result of the interaction between flavonoids and bacterial DNA [12].

The antibacterial mechanism of action of tannins has antibacterial power with how to precipitate protein. The antibacterial effect of tannins through reaction with cell membranes, inactivation of enzymes and inactivation of the function of genetic material. The mechanism of action of tannin as an antibacterial is to inhibit enzymes reverse transcriptase and DNA topoisomerase so that bacterial cells do not can form [13]. Natural tannin from the phenolic acid group and consists of a central glucose unit and ten gallic acid molecules attached to it is tannic acid. Tannic acid has many unique properties. It has anti-mutagenic and anti-tumor properties. Tannic acid shows activity against microorganisms (bacteria and viruses). It also acts as an antioxidant and homeostatic agent. In addition, tannic acid can eliminate free radicals that cause various diseases such as allergies, diabetes, Parkinson's, Alzheimer's and heart diseases. Tannic acid has also been shown to have anti-cancer activity. Now there's also tannic acid It has been studied as an organic polymer additive because it exhibits bioactive properties and improves material properties for medical applications. Therefore, it is an interesting active compound that can be used as an ingredient in foods and various consumer goods [14,15,16,17,18,19,20,21,22,23].

4. Conclusions

In conclusion, the inhibition zone data of *Grona triflora* extract and *Phyllanthus niruri* extract are called to have the similar level of sensitivity when compared with several types of antibiotics ciprofloxacin and tetracycline as positive control which have the sensitive level ≥ 21 mm.

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