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Research Article

Study The Antibacterial Activity Of Uwi Sap (Dioscorea Alata.L) Extracts From Indonesia In Damaging Escherichia Coli And Staphylococcus Aureus Cells.

A. NURFITRIANI[1*], META MAHENDRADATTA[2], AMRAN LAGA[2], ZAINAL[2]
[1]Agriculture Study Program, Graduate School Hasanuddin University Makassar 90245 Indonesia
[2]Department of food sains & Technoology, Hasanuddin University Makassar 90245 Indonesia
* Corresponding Author
E-mail: andinurfitriani87@gmail.com
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ABSTRACT

Some antibiotics are no longer effective in the treatment of infection because of resistance to microorganisms. The search for new, more effective antimicrobials from plants becomes necessary to continue to do so, especially those derived from natural ingredients. This study aims to determine the potential activity of the uwi plant (Dioscorea alata L) as an antibacterial agent for Staphylococcus aureus and Escherichia coli to its maturity. Testing to see the antibacterial inhibition was carried out using the agar diffusion method. The antibacterial mechanism was carried out using Scanning electron microscopy (SEM). The test results showed that the uwi sap extract had antibacterial activity against Staphylococcus aureus and Escherichia coli, which was characterized by an inhibition zone. The S. aureus bacteria gave the highest inhibition zone at a concentration of 96% with a diameter of the inhibition zone of 11.8 mm, while for E. coli, bacteria gave the highest inhibition zone at a concentration of 50%, namely 11.4 mm. These results also showed that the uwi sap extract could damage morphology and cause leakage of S. aureus and E. coli bacterial cells. So it can be concluded that uwi sap extract has antibacterial activity because it can inhibit the growth of S. aureus and E. coli bacteria.

Keywords: uwi sap extract, antibacterial, S. aureus, E. coli, SEM

INTRODUCTION

Treatment of infection can be treated with drugs made of chemical substances and this is not always effective, for example, treatment of infection with using antibiotics. Some antibiotics are no longer useful for therapy infection because there has been resistance to microorganisms, but it also can cause various side effects that are detrimental to sufferers. Therefore the search for new, more effective antimicrobials from plants became it is necessary to continue to do so, especially those derived from natural ingredients. The use of plants as antibacterial agents can be developed because apart from being a safer relative, the risk is also minimal compared to drugs or chemicals. Uwi plants (Dioscorea alata L) can produce tubers that asre classified by air bulbs and tubers found in the soil. Dioscorea's plant genus has 600 species spread throughout the world [1]. Uwi tubers grow using vines and include monocot plants. Purple uwi tuber contains anthocyanins and antioxidants besides that, and it has a reliable gel/sap. The gel/sap in these tubers was identified to contain secondary metabolic components. Secondary metabolite compounds found in plants have antibacterial activity, which can inhibit bacterial growth using a synergistic mechanism. Many parts of plants that contain secondary metabolites have been reported, including the presence of phytochemicals, phenols, and flavonoids in the fruit of harendong (Melastoma of ne D. Don) [2]. Phenolic components in the ethanol extract of akway bark [3,4] Flavonoids in akway [3] (Pladio & Villasenor, 2004). Flavanoid and phenolic phytochemicals are found in Dioscorea alata tubers and leaves. L [1]. Phenolic components, flavonoids in teak leaves (Tectona grandis) as antimicrobials and antioxidants [5]. Secondary metabolite components in plants act as antimicrobials [6,7,8]. Flavonoids and phenols are secondary metabolic components found in green plants and are generally found in the leaves, stems, and fruit [9]. Until now, the use of sap from uwi tubers as an antibacterial activity for S. aureus and E. coli has yet to be implemented. Therefore, this study aims to examine the activity of uwi sap extract as an antibacterial, which can inhibit the growth of E.coli and S.aureus bacteria.

MATERIAL AND METHODS

Materials

The material used is the extract of uwi sap extracted at the Pharmacognosy Laboratory, Faculty of Pharmacy, Hasanuddin University Air. Other ingredients are distilled, aluminum foil, pure cultures of Escherichia coli and Staphylococcus aureus bacteria, 70% ethanol, cotton, Nutrient Agar (NA) medium, piper disk (oxoid).

Equipment

The tools used in this study were a maceration tool, autoclave (SMIC model YX-28 B), rotavator tool, blender, petri dish (Iwaki pyrex), 250 ml Erlenmeyer (Pyrex), 100 ml measuring cup (Pyrex), incubator (Memmert), calipers, gas stove, Laminar Air Flow (LAF), spirit lamps, round loop, oven (Memmert), iron spoon, 1 ml spoit (One Med), 10 ml spoit (One Med), analytical scale (AND), test tubes (Pyrex), and vials.

Preparation of Test Bacteria

The test bacteria used in this study include Escherichia coli and Staphylococcus aureus. Bacteria originating from Hasanuddin University Medical Laboratory were rejuvenated in oblique Nutrient Agar (NA) medium and incubated for 1x 24 hours at 37°C.

Antibacterial activity test

The test method was carried out by the disk diffusion method (Salie et al., 1996; Ncube et al., 2008). The culture of S. aureus and E. coli tested bacteria aged 18-24 hours was taken 1 ose needle and smeared evenly into the NA medium using a sterile cotton swab. Furthermore, paper disk (paper disk) is sized in diameter of 6.6 mm and infused into the extract concentration of purple uwi sap (0.01%). The impregnation process was carried out by dropping 25 µL of purple uwi sap extract solution on disc paper. Disc paper containing the extract was affixed to the medium's surface so that the NA was in a petri dish. It was then incubated at 37°C for 24 hours. Measurement of the diameter of the formed zone uses a caliper.

The mechanism of microbial cell wall damage using a Scanning Electron Microscope (SEM)

The pure bacterial cell suspension treated with uwi sap extract was put into a 2% glutaraldehyde fixation solution for 2-3 hours at 4oC, then centrifuged at low speed for 15 seconds. Washed with buffer phosphate solution pH 7.4 for 3 times each for 5 seconds at 4oC, then centrifuged at low speed for 15 seconds. Post fixation with 1% osmium tetraoxide solution for 1-2 hours at 4o C, centrifuged at low speed for 15 seconds. Washed with a buffer phosphate solution pH 7.4 for 3 times each for 5 seconds at 4o C, then centrifuged at low speed for 15 seconds. Dehydration with graded ethanol: 30%, 50%, 70%, each for 15-20 minutes at 4° C, then centrifuged at low speed for 15 seconds. Next, dehydration with 80%, 90% ethanol twice, each for 15-20 minutes at room temperature, then centrifuged at low speed for 15 seconds. They were replaced with amyl acetate absolute. The bacteria to be dried are piped and dropped on a glass object with an area of 16 mm2 that has been cleaned with alcohol—drying with a Critical Point Drying (CPD) tool. Attachment to the stub (holder) using special glue. Coating with a vacuum evaporator and the coating material thereof is pure gold or carbon, the sample was observed and photographed on Scanning electron microscopy (SEM)

RESULTS AND DISCUSSION

The result of the antibacterial activity of uwi sap extract against E. coli

In this study, it was reported that the uwi sap extract samples gave E. coli antibacterial activity. This was indicated by a clear zone visible after administration of uwi sap extract (Figure 1). Based on the results of measurements, The diameter of the sample's resistance is apparent that each concentration of the sample gives a different size of the resistance diameter. Activities are given Uwi sap extracts at a concentration of 96% v / v resulted in the highest inhibition zone diameter of 11.8 mm (Figure 1), while the lowest inhibition zone was at the concentration of ethanol 65% v / v solvent of 9.9 mm (Figure 1). In drug control, using positive tetracycline control resulted in inhibition with a diameter of 25.5 mm.

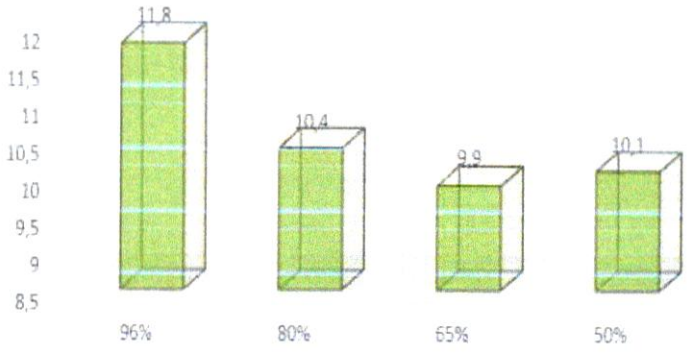

Fig.1: The measurement of the inhibition zone diameter (mm) of ethanol extract of uwi sap was 96%, 80%, 65%, and 50% of the Escherichia coli bacteria activity.

This proves that the higher the concentration of a compound, the higher the activity. This is due to certain chemical compounds that are thought to be contained in the sap extract sample uwi, which is antibacterial. The difference in diameter can be influenced by the type of test bacteria used. Each bacteria has a different sensitivity to the sample, in this case, the antibacterial compound, where a bacterium will forming resistance in itself, which is a natural mechanism for survival (Mutscler, 1991). Apart from the influence of the type of bacteria, differences. The inhibition diameter is also due to the sample's concentration, in this case, the ability of the substances that are thought to be contained in the sample to inhibit the growth of the tested bacteria. This research report provides the most recent information that uwi sap extract can provide antibacterial activity and can be used as an antibacterial candidate in the future.

The results of the antibacterial activity of uwi sap extract against S. aureus

In this study, it was reported that the uwi sap extract provided antibacterial activity against S. aureus. This was evidenced by the resulting inhibition zone, which was marked with a clear area. The formation of an area of inhibition around the paper disk shows antibacterial compounds against the test bacteria, namely S. aureus. The wider the zone of inhibition that is formed many dead bacteria can be seen from the exact area around the paper disk (Figure 2)

Uwi sap extract at 96% concentration yielded an inhibition zone diameter of 9.6 mm (Figure 2). This result is the smallest inhibition zone in S. aureus. The activity of the largest inhibition zone given by uwi sap extract was at a 50% concentration, which is equal to 11.4 mm. Meanwhile, the control drug tetracycline provides antibacterial activity against S. aureus by 36 mm. The difference in zone diameter inhibition that occurs explains that the difference in concentration can affect the diameter of the zone of inhibition.

These results prove that the uwi sap extract with ethanol solvent can affect S. aureus bacteria's development so that the uwi sap extract can be used and developed as an antibacterial.

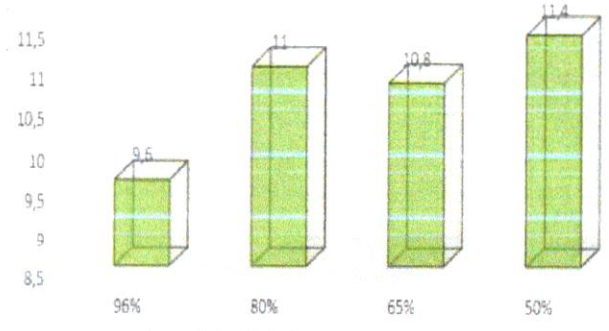

Fig.2: The measurement results of the inhibition zone diameter (mm) of ethanol extract 96%, 80%, 65%, and 50% against the activity of Staphylococcus aureus bacteria with a minimum concentration of 0.01% uwi sap extract.

Changes in cell wall morphology of E. coli bacteria after the addition of two sap extracts.
E. coli Gram-negative bacteria cells have short rods' characteristics, measuring 0.4-0.7 nm x 1.4 nm, growing in the range 20-40 ^{0}C, optimum at 37 ^{0}C. E. coli is about 2 micrometers in size and 0.5 in diameter micrometers. The volume of E. coli cells ranged from 0.6 to 0.7 cubic micrometers.

Changes in the morphology of bacterial cells through scanning electron microscope (SEM) observation in Figure 3, after the addition of 0.01% sap extract resulted in damage and leakage of cells and cells. Bacteria are not straight rods, and there are indentations (Figure 3).

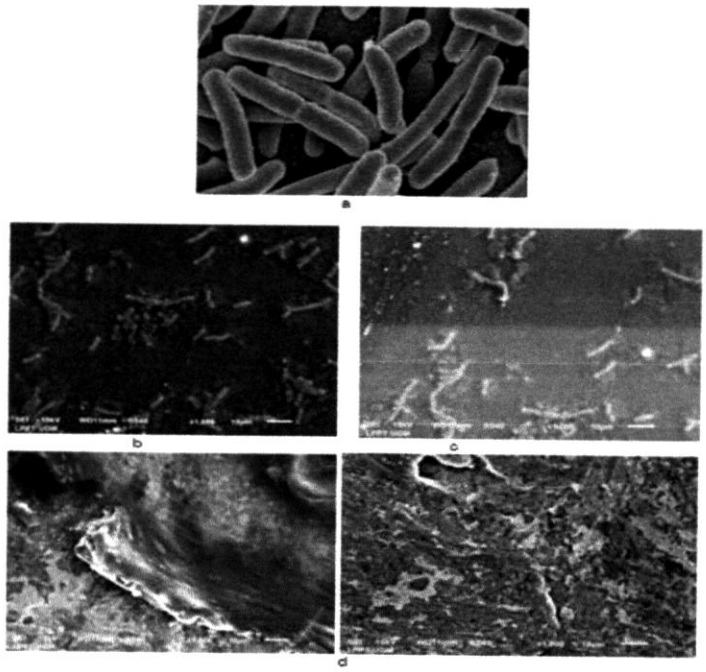

Fig.3: Changes in E. coli morphology due to exposure to uwi sap extract. a) The appearance of E. coli cell morphology. b) E. coli bacterial cells change shape after adding sap extract, c) E. coli cells experiencing cell shape indentation, d) E. coli cells experience cell wall leakage.

Changes in E. coli cells' morphology can be caused by the presence of phenol, which causes the bacterial cell membrane to undergo lysis due to protein coagulation [10,11]. When the active substance (antibacterial) reacts with the sides active of the membrane and increases its permeability, it can damage bacterial cell membranes. Flavonoid compounds cause cell protein to experience clotting. So that there are denaturation and protin becomes inactive [12]. Flavonoids in high concentrations cause total damage to bacterial cell membranes and precipitate cell proteins. In contrast, in low concentrations, they cause leakage of bacterial cells to release metabolites - essential metabolites of bacterial cells [13]

Changes in cell wall morphology of S. aureus bacteria after the addition of uwi sap extract.
The analysis of microbial cell damage analysis using SEM was carried out based on the sample that had the highest minimum inhibition zone power. Staphylococcus aureus normal cells have a size of 0.5 to 2.0 nm. cells are round and are grouped as shown in Figure (4)
The effect of adding 0.01% uwi sap on bacteria S. aureus can be seen in Figure 8, resulting in damage to the cell wall where in the picture shows the cell changes shape, the cell leaks and is irregular.

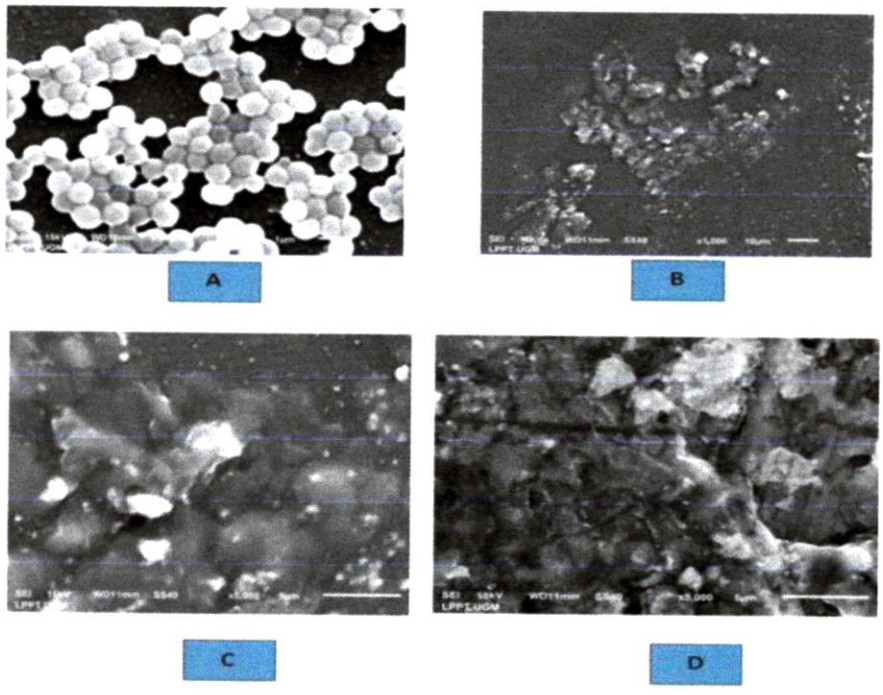

Fig.4: Morphological changes of Staphylococcus aureus due to exposure to uwi sap extract. a) The appearance of S. aureus cell morpholog. b) bacterial cells change shape after adding sap extract, c) S. aureus has an indentation of cell shape, d) S. aureus cells leak cell walls.

Cell membrane damage can occur when active antibacterial compounds react with the membrane's operational side or by dissolving lipid constituents and increasing their permeability. The bacterial cell membrane is composed of phospholipids and protein molecules. With an increase in permeability, antibacterial compounds can enter the cell and lyse the cell membrane or coagulate the bacterial cell [14,15]. Phenol causes the bacterial cell membrane to undergo lysis due to protein coagulation (Parwat et al. 2008), flavonoids damage bacterial cells [16]. Flavonoids can also inhibit the bacterial DNA gyrase enzyme [17]. The DNA gyrase enzyme plays a role in opening the DNA strands for the DNA replication process. If the DNA gyrase enzyme is inhibited, the DNA replication process and transcription are inhibited, resulting in damage to bacterial cells and, ultimately, bacterial cell death [18].

CONCLUSION

Uwi sap extract can provide the antibacterial activity of S. aureus and E. coli. It can damage the cell morphology of S. aureus and E. coli bacteria and cause cell leakage. Therefore Uwi sap extract has the potential to be developed as a new antibacterial drug.

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Competing Interests

The authors declare no competing interests.

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