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Antibacterial Effectiveness of Purple Yam (*Dioscorea alata* L) Sap Extract in Inhibiting the Growth of *Staphylococcus aureus* & *Escherichia coli*

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ABSTRACT

Purple yam sap extracts have secondary metabolite components which can be utilized as an antibacterial property. Secondary metabolite compounds found from the result of the preliminary study include saponins, tannins, flavonoids, phenols, alkaloids, and triterpenoids. The aim of this study was to analyze the antibacterial activity of sap extracts towards the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria. The maceration method of 96% ethanol as a solvent was used to obtain purple yam sap extracts with a 48-hour maceration time. The effectiveness of bacterial inhibition zones based on the minimum inhibitory concentration (MIC) was carried out by testing eight concentrations of 0.01%, 0.02%, 0.04%, 0.06%, 0.08%, 0.15%, 0.20%, and 0.25% with tetracycline as a positive control and dimethyl sulfoxide (DMSO) as a negative control. The results showed that the minimum inhibition of purple yam sap extracts with the 0.01% concentration inhibited the growth of gram-positive *Staphylococcus aureus* bacteria with a medium category of inhibition zone of 8.62 mm. On the other hand, gram-negative *Escherichia coli* bacteria form a minimum inhibition zone of 9.30 mm. The maximum extract concentration in inhibiting the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria was 0.25% (21.77 mm,13.35 mm), which was categorized as the strongest inhibition zone for bacterial growth. Accordingly, purple yam sap extracts macerated with 96% ethanol during the 48-hour period with 0.01% extract concentration were effective in inhibiting the growth of gram-positive bacteria (*Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli*).

Keywords: *Antibacterial, Secondary metabolite, Purple yam.*

1. INTRODUCTION

Yams (*Dioscorea*spp) are able to grow in tropical and subtropical regions [1]. More than 600 species from the *Dioscorea* genus spread in various countries, including Indonesia, and some of which are *Dioscoreaalata* L (purple yam, coconut yam, *keribang*, and water yam), *Dioscoreaesculanta* (lesser yam), Dioscoreaopposite (white yam), *Dioscoreaavillosa* (yellow yam), *Dioscoreahispida* (Indian three-leaved yam), and *Dioscoreabulbifera* (true yam/air potato) [2]. Apart from being a food crop, purple yam can be potentially utilized as traditional medicine. Purple yam has sap/mucus that contains phytochemical compounds, and potentially serves as an antimicrobial aiming to control the growth of harmful bacteria and further kill the microbes [3]. The substances prevent bacterial growth by inhibiting the formation process, resulting in changes in the permeability in the bacterial cytoplasmic membrane.

The sap in purple yam contains mannans and proteins (5-10%) that can affect the physicochemical properties of purple yam [4]. The sap on the surface of

purple yam contains alkaloid compounds as well as polysaccharides that form thick colloids [5]. Thick sap consists of polysaccharides (mannan and cellulose) and glycoproteins [6]. Meanwhile, the polysaccharides in purple yam consist of glucose, mannose, arabinose, galactose, rhamnose, and xylose [7].

The phytochemical components found in purple yam sap as based on the result of the preliminary study are alkaloids, tannins, saponins, flavonoids, phenols, glucomannans, and glycoproteins. In several studies, it is also shown that purple yam comprises flavonoids, phenols, terpenoids, tannins, saponins, alkaloids, polysaccharide groups, cardiac glycosides, organic acids, and oxalates [8] [9] [10].

The secondary metabolite components found in plants are alkaloids, terpenoids, tannins, and phenolics [11]. Further, plants containing saponins, phenols, flavonoids, tannins, alkaloids, terpenoids, sesquiterpenes, phorbol esters, glycoalkaloids, and lactones serve as antimicrobial agents [12]. The parts of plants that have secondary metabolite components can potentially be used as antimicrobials [13] [14].

Based on the study of the contents of purple yam sap components, it is hypothesized that this plant is effective in inhibiting bacterial growth or serving as an antibacterial in gram-positive and gram-negative bacteria.

2. MATERIAL AND METHODS

2.1 Purple Yam Types

The raw material in this study was an 8-month-old purple yam obtained from the Enrekang Regency of South Sulawesi.

2.2 Purple Yam Sap Extracts

Purple yam sap extracts was obtained using the purple yam maceration method [16]. As many as 500 grams of purple yam were macerated for three days using a single solvent (ethanol 96%). The sap extraction was done by vacuum filtering employing the Whatmann paper No. 1. The obtained filtrate was then evaporated using a rotary evaporator at a temperature of 60-75°C for 30 minutes. The sap extracts were then placed into a desiccator.

2.3 Secondary Metabolite activities

An analysis of secondary metabolite activities in purple yam sap was carried out by an identification using thin-layered chromatography (TLC) [16]. The thin-layered silica gel plate was made into a size of 6x7 cm, and then the sample of purple yam sap extract was bottled into a thin layer with a capillary tube. The solvents used were butanol, glacial acid, sterile aquadest (1: 0,5: 0,5). The plate was then put into a chamber that had been filled with the solvents. After it dried, the plate was taken out from the chamber and dried with a vacuum dryer. An observation of the formed stains used the UV light 254 nm and UV 366 nm. Spraying plates with FeCl3 solvents (tannin, phenol), cytoborates (flavonoids), cerium sulfate (alkaloid), steroids (Liebermann Burchard), saponins (vanillin) was carried out. The plate was then heated until the color changed, and the color identification was then performed.

2.4 Antimicrobial Activities

The measurement of antimicrobial activities was done by using the agar diffusion method [17] [18]. The bacterial isolates encompassed *Escherichia coli* and *Staphylococcus aureus* bacteria. One needle of bacterial suspension was inserted into the Mueller-Hilton sterile media in laminar flow. A paper disk was impregnated / hatched with 0.25 ml of purple yam sap extracts based on concentrations of 1%, 2%, 4%, 6%, 8%, 15%, 20%, 25%. Moreover, the paper disk was inserted into Petri dishes that had been applied to bacterial isolates, in which the positive control used DMSO (dimethyl sulfoxide) and the negative control utilized tetracycline. Petri dishes containing extracts and bacteria were incubated at 37°C for 18-24 hours. After 24 hours, a clear zone around the medium was measured using the calipers.

3. RESULT AND DISCUSSION

3.1 The yield of yam sap extracts and secondary metabolite components

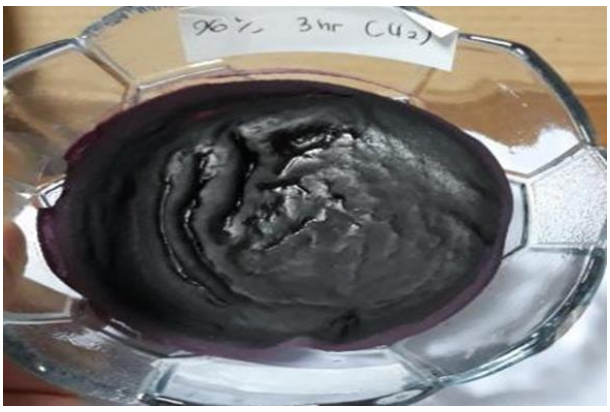

Figure 1. The extracts of purple yam sap extracted by ethanol 96%

Table 1. Identification of active components in sap extracts.

Types of active substances	Reagents	Stain colors	Identification
Alkaloids	Dragendorff reagent	Brownish-yellow	+
Phenols	FeCl3	Bluish-black	+
Flavonoids	Sitoborat	Green fluorescence	+
Tannins	FeCl3	Bluish-black	+
Saponins	Liebermann Burchard	Reddish-purple	+
Steroids/ triterpenoids	Vanilinsulfuric acid	Red, bluish-purple	+

Table 2. The diameters of the inhibition zone of the *Escherichia coli* growth.

Sap extracts (Ethanol 96 %) Concentration	negative control (DMSO)	Gram bacteria (Escherichia coli)	inhibition zone category
1 %	6.6 mm[a]	9.3 mm	Moderate
2 %	6.6 mm[a]	8.4 mm	Moderate
4 %	6.6 mm[a]	9.85 mm	Moderate
6 %	6.6 mm[a]	10.9 mm	Strong
8 %	6.6 mm[a]	10.05 mm	Strong
15 %	6.6 mm[a]	11.85 mm	Strong
20 %	6.6 mm[a]	13 mm	Strong
25 %	6.6 mm[a]	13.35 mm	Strong

[a] paper disk diameter of 6.6 mm

The yield of purple yam sap extracts is accounted for 62,03%. Since ethanol as the solvent has a high polarity index, it is able to dissolve some active substances in the ingredients. Concentrated extracts resulted from the evaporation process contain polar compounds in the form of alkaloids, flavonoids, phenols, steroid saponins, and tannins.

Table 1 presents the secondary metabolite components extracted as antibacterials. The identification of the active substances utilizes the TLC method (figure 2), where the separation of the active substance components (mobile phase and stationary phase) is based on the level of polarity. Therefore, there is a separation of the active ingredients in the extracts. The extracts on the TLC profile use 0.5 ml of glacial acid (elution) mobile phase: 1 ml of butanol: and 0,5 ml of distilled water.

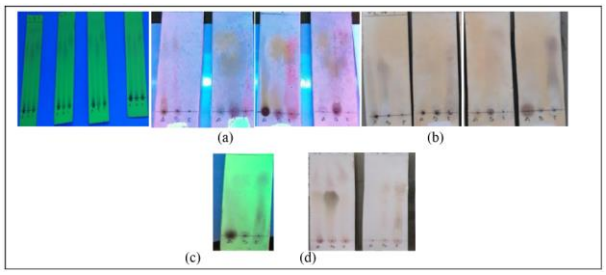

Figure 2. TLC profile of sap extracts with some formulations (a) vanillin sulfuric acid, (b) Fecl3, (c) Cytoborate, (d) Liebermann Burchard.

The results obtained by using 254 nm UV light after the process of spraying and heating with the vanillin sulfuric acid reagent create a red, purple, and bluish colors. This shows that the color changes are the identification of the presence of active substances in the form of steroids and triterpenoids in the sap extracts. A study conducted by [19] reports that steroid identification is marked by changes in the color of the solution when adding concentrated sulfuric acid and anhydrous acetic acid. [20] also state that color changes in the identification of triterpenoid substances include orange-red to purple if they contain blue steroids.

$FeCl_3$ is a typical reagent utilized to detect the presence of tannin and phenolic compounds. Based on the analysis, black and blue stains are formed during the process of spraying and after heating on the KLT plate that contains the extracts. These color changes identify the presence of phenol/polyphenol/tannin compounds. Color changes with the addition of Fecl3 will cause blackish or greenish-black, and blue colors [21].

The identification of the flavonoid component by editing the cytoborate under UV light 254 shows a glowing green. According to [22], the presence of flavonoid compounds is characterized by changes in greenish-yellow color after being sprayed with cytoborate reagent. The reagent of the Liebermann Burchard is used to detect the presence of saponins contained in the extracts which are characterized by the formation of purple color after the spraying process. Besides, Wagner and Baldt (1996) point out that spraying with the Liebermann Burchard experiment shows saponins.

3.2. Inhibition zone activity

3.2.1. Escherichia coli.

The results indicate that purple yam sap extracts can inhibit the growth of *Escherichia coli* (gram-negative bacteria), proven by the formation of the inhibition zone around the medium that contains bacterial cultures and sap extracts.

The results of the study (Table 2) regarding the use of extracts at concentrations of 1% to 25% inhibit the *Escherichia coli* bacteria. The use of negative control of dimethyl sulfoxide (DMSO) has no effect on bacterial inhibition. In addition, the effect of concentrations for the inhibition zone is based on the diameter, in which the greater the extraction concentration, the larger the diameter of the inhibition zone. The concentration of 1% extract forms 9.3 mm in diameter, and includes in the medium category. However, the extract of 25% concentration forms 13.35 mm in diameter and is categorized in the strong category. This is based on a study by [24] signifying that determining the category of bacterial inhibitory strengths is based on the size of the formed diameter. The diameters of ≥ 20 mm, 10-20 mm, 5-10 mm (inhibition zone), and 5 mm include in the very strong, strong, moderate, and weak categories, respectively.

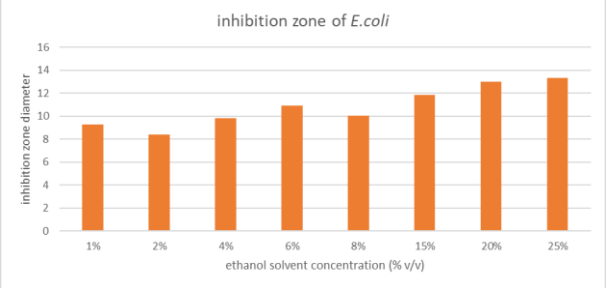

Figure 3. The Effect of extract concentrations on the inhibitory power on *Escherichia coli* bacteria.

The testing results of the effect of extraction concentration (figure 3) show that a low concentration of 1% will give a small inhibitory power of 9,3 mm; the extract with a concentration of 25% forms a significantly greater zone of 13,35 mm%. This is due to the fact that the greater the concentration, the higher the number of components of the active/metabolite substances contained in the extracts. This is supported by [25] noting that the high concentration of ingredients will result in higher antimicrobial inhibition.

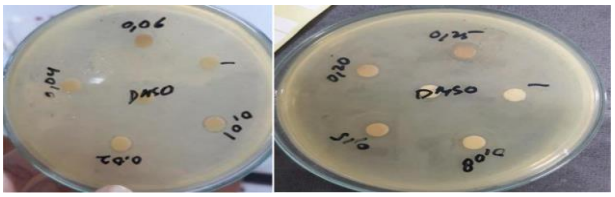

Figure 4. The inhibition zone formed in the *Escherichia coli*

Antibacterial activities against *Escherichia coli* bacteria (figure 4) are influenced by the concentration of the material. The high extracts lead to a large diameter in the media. Active ingredients in the extracts can function as antibacterials. Additionally, the secondary metabolite components that have been identified utilizing the TLC profile are phenols, tannins, steroids/saponins, and flavonoids, which serve as active substances that inhibit bacterial growth. Phenols cause bacterial cell membranes to be lysed due to protein coagulation [26]; tannins can activate microbial cell enzymes [27]; steroids result in damaged microbial cell plasma membranes [28]; flavonoids damage bacterial cells [29]; and alkaloids inhibit wall synthesis cell [30].

3.2.2. Staphylococcus aureus.

The inhibition zone of the *Staphylococcus aureus* bacteria (Table 3) shows that the concentration variations give different formations of inhibitory diameter. The negative control of dimethyl sulfoxide (DMSO) which was used as a solvent for making concentration variations has no antibacterial activities in

order that the activities only come from the test solution.

Following the results of the study on Table 3, the extract concentration of 1% leads to the diameter of the inhibition zone of 8.62 mm with the moderate category of inhibition. Nevertheless, the extract concentration of 25% forms an inhibition zone of 21,77 mm and falls under a very strong category. In this case, the formed inhibition zone is one of the bacterial responses to purple yam sap ethanol extracts as antibacterial agents. [31] state in their study that ethanol extracts containing alkaloids, flavonoids, saponins, and tannins have the greatest ability to inhibit the growth of gram-positive *Staphylococcus aureus* bacteria. In the same tune, [32] also describes that the best antibacterial activities against gram-positive bacteria are found in the ethanol extracts from the vegetable hummingbird.

Table 3. Diameter of inhibition zone of *Staphylococcus aureus* growth

Sap extracts (Ethanol 96 %) Concentration	Negative control of dimethyl sufoxide (DMSO)	Gram bacteria(+) *Staphylococcus aureus*	Inhibition zone category
1 %	6.6 mm[a]	8,62 mm	Moderate
2 %	6.6 mm[a]	10,25 mm	strong
4 %	6.6 mm[a]	11,12 mm	strong
6 %	6.6 mm[a]	10,67 mm	strong
8 %	6.6 mm[a]	10,90 mm	Strong
15 %	6.6 mm[a]	18,81 mm	Strong
20 %	6.6 mm[a]	20,93 mm	Very Strong
25 %	6.6 mm[a]	21,77 mm	Very strong

[a] paper disk diameter of 6.6 mm.

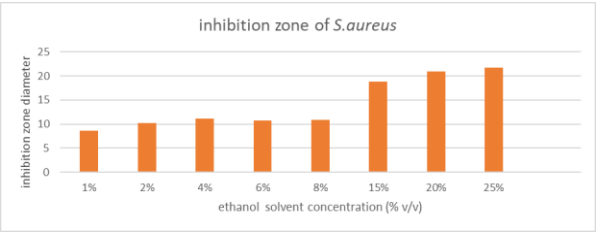

Figure 5. The effect of extract concentrations on the inhibition of *Staphylococcus aureus*

Figure 5 reveals that the extract concentration of 25% has a very strong inhibitory activities compared to the inhibitory power formed in gram-negative *Escherichia coli*, implying that the gram-positive *Staphylococcus aureus* bacteria is more sensitive to antibacterial substances than *Escherichia coli* bacteria. The structure of the cell walls of each bacterium causes different levels of sensitivity. One gram-positive *Staphylococcus aureus* has two layers of cell wall, meanwhile, the *Escherichia coli* has a more complex cell wall layer. For this reason, the extract components diffuse more easily into the cell wall of *Staphylococcus aureus* bacteria. [33] opine that *Escherichia coli* bacteria have bilayer outer membranes, lipids, and peptidoglycans.

The observation indicates that the diameter of the inhibition zone (figure 5) is the sensitivity response of the *Staphylococcus aureus* bacteria to the given amount of antibacterial concentrations. Antibacterial concentrations, incubation temperature, pH of the media, and the number of inoculums are factors that influence antibacterial activities. Additionally, differences in the active substance components in the extracts also cause differences in the length of the diameter of the inhibition zone. This is in accordance with [34] claiming that the size of the inhibition zone is affected by several things, such as antibacterial compounds, concentrations of antibacterial compounds, the sensitivity of organisms, and the speed of diffusion extracts.

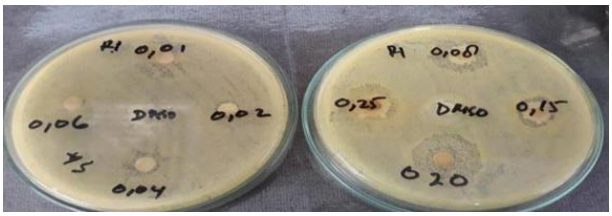

Figure 6. Inhibition zone formed in *Staphylococcus aureus*

4. CONCLUSION

This study concludes that the purple yam sap extracts have effective components to inhibit bacterial growth. The minimum inhibition zones of the *Escherichia coli* and *Staphylococcus aureus* bacteria with a concentration of 1% form the diameter of 9.3 mm and 8.62 mm, respectively. Each inhibition zone is a moderate inhibition with the smallest concentration of 1%. The very strong inhibition, in contrast, is at the concentration of 25%. Moreover, ethanol extracts in purple yam sap have very strong inhibitory activities in gram-positive bacteria, compared to the gram-negative bacteria.

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