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# TESTING THE RESPONSIBILITY OF EGGPLANT THORN (SOLANUM CAROLINENSE) ETHANOL EXTRACT ON THE GROWTH OF BACTERIA (STREPTOCOCCUS MUTANS) IN VITRO

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***Abstract***

*Streptococcus mutans is the main pathogenic bacteria causing caries and tooth enamel damage. Eggplant spines (Solanum carolinense) contains active substances that can inhibit the growth of Streptococcus mutans bacteria on teeth. The purpose of this study was to determine the inhibitory effect of the antibacterial activity of ethanol extract of thorn eggplant on the growth of Streptococcus mutans bacteria and to determine the optimal concentration of thorn eggplant extract on the growth of Streptococcus mutans bacteria in vitro. The type of method used in this study is an experimental study, with the dependent variable being the zone of inhibition of the growth of Streptococcus mutans, the independent variable being differences in the concentration of ethanol extract of thorn eggplant (Solanum carolinense) at concentrations of 20%, 40%, 60%, 80% and 96 ethanol. % as negative control and chloramphenicol as positive control, control variables for the presence of other microbial contamination, media thickness, turbidity of bacterial suspension, incubation temperature, disc distance, incubation time, sterilization of equipment, media and room. The results of this study were the effect of the ethanol extract of thorn eggplant on Streptococcus mutans and the optimal concentration of the ethanolic extract of thorn eggplant was 80% with an inhibition zone diameter of 11 mm (strong) which was indicated by the appearance of the diameter of the bacterial inhibition zone at each treatment.*

***Keywords**: Inhibitory Test, Extract, Eggplant Thorns, Streptococcus mutans, In Vitro.*

## INTRODUCTION

Infectious diseases are among the most important health problems in developing countries, including Indonesia. One of the most common infectious diseases is a disease caused by bacteria. Bacteria are one of the microbes that are the leading cause of pathogenic diseases worldwide. One bacteria that causes the condition is Streptococcus mutants (Novani, 2019). This bacterium is a pathogenic bacteria in the mouth due to the high humidity, the constant presence of dissolved food, and the tiny particles of food that make the mouth an ideal environment for the growth of Streptococcus mutans bacteria. Andries et al., 2014 stated that Streptococcus mutans bacteria only had a positive correlation with the presence of caries on the tooth surface. The Basic Health Research (2013) results show that the national prevalence of dental and oral problems is 25.9%. As many as 14 provinces have most dental and oral issues than the national figure (Depkes RI, 2013). Dental caries is a dental disease that can interfere with daily activities. Foods that contain sugar are one of the triggers for dental caries. Children very much like foods containing sugar. Therefore the highest prevalence of dental caries sufferers is in children. Dental caries is treated using antibiotics (Koswara, 2007).

Antibiotics are often used to treat dental caries. Even all hospital patients get antibiotics. However, the many cases experienced severe side effects, namely resistance to antibiotics (Mandal et al., 2008). Therefore, it is necessary to have traditional medicine as alternative medicine that is cheap, easy to obtain and accessible to the general public, and useful for inhibiting or killing bacteria in the mouth. One of them uses natural ingredients, namely thorn eggplant (Solanum carolinense).

According to (Culbreth, 1927; Utami, 2013), the thorn eggplant Solanum carolinense contains active ingredients, including glycoalkaloids (alkaloids + sugar) in the form of solanine and solanidine. Fruit extracts have been shown to have antibacterial and antifungal properties, although the active constituents of the sections are unknown (Reynolds, 1990). Therefore, researchers suspect that this alkaloid content can inhibit antibacterial activity. Then from previous research on "Ethnobotany studies of medicinal plants in the community of Arjasa sub-district, Kangean Islands, Sumenep Regency, Madura." The results showed that the percentage of the use of thorn eggplant (Solanum carolinense) for toothache was 0.43% (Sofiah, 2014).

Based on this background, it is necessary to conduct a study to obtain a theoretical basis and scientific evidence regarding the efficacy of thorn eggplant (Solanum carolinense). In this study, researchers will examine the inhibition of thorn eggplant extract against Streptococcus mutant bacteria in vitro laboratory studies.

## METHODS

This research is an experimental study by testing the inhibitory power of ethanol extract of thorn eggplant (Solanum carolinense), using control samples with 20%, 40%, 60%, and 80%. The negative control was made by immersing the disc in 96% ethanol. The positive control was the antibiotic chloramphenicol disc. In this study, the use of positive control was only used as a work control.

## RESULT AND DISCUSSIONS

**Table 1.** Results of Phytochemical Screening Test for Eggplant Durian Ethanol Extract

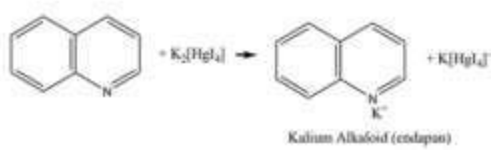
| Parameters | Reagent | Sample | Change |
|---|---|---|---|
| Alkaloid | Mayer | Positive | Fog |
| | Dragendrof | Negative | Sediment White |
| | Bouchhardat | Positive | Sediment Chocolate |
| Flavonoid | Etil asetat&FeCl3 | Positive | Black |
| Saponin | Aquadest | Positive | foam |
| Terpenoid | CH3COOH | Positive | purple |

Phytochemical screening is a preliminary test of Simplicia materials that aims to determine the class of chemical compounds in plants. Phytochemical screening includes qualitative analysis of plants or plant parts (roots, stems, leaves, flowers, fruits, and seeds), especially the secondary metabolites, bioactive compounds such as alkaloids and flavonoids, glycosides, terpenoids, tannins, and saponins. The method used in the phytochemical screening process must meet the following requirements: simple, fast, can be carried out with minimal equipment, selective for the class of compounds being studied, and can provide additional information on the presence or absence of certain compounds from the type of compounds being reviewed. Based on Table 1, screening results Phytochemicals of the ethanolic extract of thorn eggplant contain a class of alkaloid compounds, flavonoids, and saponins. This is because ethanol is a universal solvent with a polar group (-OH) and a non-polar group (-CH3) to attract polar and non-polar analytes.

Alkaloids analysis used 3 mL of extract, then added ten drops of 2N HCl. In identifying the group of alkaloid compounds, the addition of acid, namely HCl 2N, because the alkaloid compounds in plants are generally found in the form of salts that are soluble in water. Then, precipitating reagents were added, namely Mayer, Dragendrof, and Wagner. With these reagents, the alkaloids will react with nitrogen to form a precipitate. The identification results showed positive results in the ethanol extract of thorn eggplant, which was indicated by the color of the solution becoming cloudy and foggy.

A positive test for Meyer's reagent is indicated by forming a white residue. It is thought that this precipitate is a Potassium-alkaloid complex. Adding HCl is because the alkaloids are alkaline, so they are extracted with an acid solvent. Treatment of the extract with NaCl before adding the reagent was carried out to remove protein. In the manufacture of Meyer's reagent, a solution of Mercury (II) chloride plus Potassium Iodide will react to form a red precipitate of mercury (II) iodide. Because potassium iodide is added in excess, it will create Potassium tetraiodomercurate (II) (Setiono, 1990). The approximate reaction of the Mayer test is shown in Figure 4.7 (McMurry, 2004).

$$HgCl_2 + 2\,KI \rightarrow HgI_2\downarrow + 2\,KCl$$
$$HgI_2\downarrow + 2\,KI \rightarrow [HgI_4]^{2-} + 2K^+$$




**Picture 1**. Mayer's Test Reagent

The formation of a light brown also indicated positive results of alkaloids in Dragendorff's test to yellow precipitate. The precipitate is a potassium alkaloid. In the manufacture of Dragendorff's reagent, bismuth nitrate is dissolved in HCl to prevent a hydrolysis reaction from occurring because bismuth salts are readily hydrolyzed to form bismuthyl ions (BiO+) as shown in Figure 4.8 For Bi3+ ions to remain in solution, acid is added to the solution so that the equilibrium will shift. to the left

$$Bi^{3+} + H_2O \longrightarrow BiO^+ + 2H^+$$

**Picture 2.** Bismuth hydrolysis reaction

Furthermore, the Bi3+ ion from bismuth nitrate reacts with potassium iodide to form a black precipitate of Bismuth (III) iodide, which dissolves in excess potassium iodide to form potassium tetraiodobismutate (Svehla, 1990). In the alkaloid test with Dragendorff's reagent, nitrogen is used to form coordinate covalent bonds with K+, which is a metal ion. The reaction in Dragendorff's test is shown in Figure 4.9 (Miroslav, 1971)

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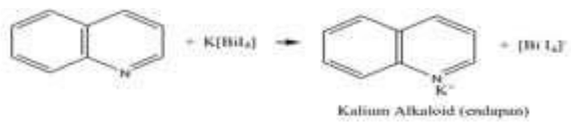
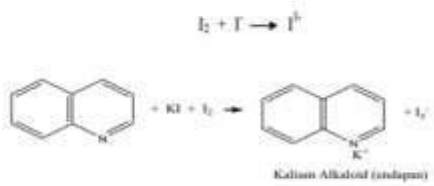
$$Bi(NO)_3 + 3\,KI \rightarrow BiI_3 + 3\,KNO_3$$
$$BiI_3 + KI \rightarrow K(BiI_4)$$



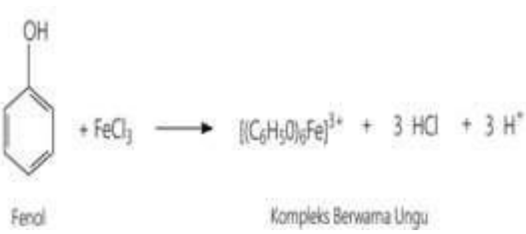

**Picture 3.** Dragendrof test reaction

On the addition of Wagner's reagent, a positive test result for alkaloids was indicated by forming a light brown to yellow precipitate. It is estimated that the deposit is potassium-alkaloids. In preparing Wagner's reagent, iodine reacts with me- ions of potassium iodide to produce I3- brown ions. In the Wagner test, metal ions K+ will form coordinate covalent bonds with nitrogen in the alkaloids to form potassium-alkaloids (Marliana et al., 2005). The reaction of the Wagner test is shown in Figure 4.10 (Marliana, 2005).




**Picture 2**. Wagner test reaction

The phytochemical test results show phenolic compounds in the ethanol extract of thorn eggplant fruit. Phenolic compounds tend to be readily soluble in polar solvents such as ethanol and water because they bind to sugars as glycosides and are usually found in cell vacuoles (Harborne, 1987). The reaction of FeCl3 with the sample causes the formation of color in this test, the role of which is the Fe3+ ion that undergoes hybridization, as shown in Figure 5.




**Picture 5.** Phenol with feCl3 (Sagar, 1996).

A black color change occurred in the flavonoid test from the thorn eggplant's ethanol extract. Flavonoids are antimicrobials that disrupt the function of the cytoplasmic membrane, and the largest group of phenolic compounds, phenolic compounds, have effective properties to inhibit the growth of viruses, bacteria, and fungi (Rahayu, 2013). Flavonoids have a mechanism of action in inhibiting bacterial growth by inactivating proteins in cell membranes. Most of the cell structure and cytoplasmic membrane of bacteria contain proteins and fats. Instability in the cell wall and cytoplasmic membrane of bacteria causes active transport, which will result in the escape of macromolecules and ions from the cell. So that bacterial cells lose their shape (Virgianti, 2015).

Saponin test on an ethanol extract of thorn eggplant fruit with the addition of aqua dest. There was a change in foam in testing the saponins from the ethanol extract of the thorn eggplant. Saponins contain glycoside groups. Glycosides are a complex between reducing sugars (glycones) and non-sugars (aglycones). Glycon is easily soluble in water. In addition, saponins are firm surface-active compounds that cause foam when shaken with water. Saponins are secondary metabolites in plants that act as antibacterial. The appearance of foam indicates the presence of glycosides which are hydrolyzed in water into glucose and other aglycone compounds (Robinson, 1995). The mechanism of action of saponins is included in the antibacterial group, which interferes with the permeability of bacterial cell membranes, results in cell membrane damage, and causes the release of various essential components from the bacterial cell, namely proteins, nucleic acids, and nucleotides. This ultimately results in bacterial cell lysis (Kurniawan, 2015).

In the terpenoid test on the ethanol extract of thorn eggplant fruit with the addition of glacial CH3COOH & 1 ml H2S04 on the terpenoid test of the ethanol extract of the thorn eggplant, there was a red ring color change. The results obtained showed positive results with the formation of a brownish-red color indicating the presence of terpenoid content (Sangi et al., 2008). Testing of steroids and triterpenoids in glacial CH3COOH with concentrated H2SO4 was based on the ability of the steroid and triterpenoid compounds to form blue or green colors for steroids and red or purple for triterpenoids. Steroids and triterpenoids are compounds extracted with non-polar or semi-polar solvents (Harborne, 1987; Nurjanah, 2011).

In this study, four concentrations of eggplant extract were tested against the growth of Streptococcus mutans bacteria, namely concentrations of 20%, 40%, 60%, and 80%, where each engagement had a different diameter of inhibition zone. Based on the measurement results, it is known that all concentrations of ethanol extract of thorn eggplant fruit that were tested against the growth of Streptococcus mutans bacteria showed a clear zone. From table 2. It can be seen that the ethanolic extract of thorn eggplant showed a diameter of inhibition zone that was more effective against gram-positive bacteria Streptococcus mutants at a concentration of 80% with an inhibition zone diameter of 11 mm.

The results of the antibacterial activity test showed that the more concentrations given, the greater the diameter of the barriers produced. Namely, from the antibacterial test results, the concentration of 20% was 7 mm (medium), 40% was 8 mm (medium), 60% was 9 mm (medium), 80% concentration is the highest concentration that can inhibit the growth of Streptococcus mutans which provides an inhibitory power of 11 mm (robust).

Based on the results of the study, it can be seen that the thorn eggplant (Solanum carolinense) fruit extract at various concentrations was able to inhibit the growth of Streptococcus mutans which was characterized by the formation of a clear zone around the disc. According to Susanto et al. (in Permadani, Puguh, and Sarwiyono, 2014), if the diameter of the inhibition zone is more than 20 mm, then the extract is categorized as having potent inhibition. If the diameter of the inhibition zone is in the range of 11-20 mm, then the section is classified as having potent inhibition, and if in the field of 5-10 mm, it is said to have moderate inhibition, whereas if the diameter of the inhibition zone is less than 5 mm, then the extract is categorized as having weak inhibition. So that the 20% concentration of thorn eggplant extract was said to have weak inhibition, 40% and 60% had moderate inhibitory power, and 80% had potent inhibition. While the control 96% ethanol has soft inhibitory power, there is no inhibition zone indicated by the absence of a clear area around the disc with a measured value of 0.00 mm. These results suggest that the control containing 96% ethanol used as a diluent did not affect the inhibition zone formed at each concentration.

The results also show that the higher the concentration, the larger the inhibition zone formed around the paper disc. This result follows the statement of Prawata and Dewi (2008) in (Roslizawaty et al., 2013) that the effectiveness of an antibacterial substance is influenced by the concentration of the essence. The increase in the concentration of substances causes an increase in the content of active compounds that function as antibacterials. Their ability to kill bacteria is also extraordinary.

## CONCLUSIONS

From the results of this research, several conclusions can be drawn:

1. The effect of thorn eggplant (Solanum carolinense) fruit extract at various concentrations

is able to inhibit the growth of Streptococcus mutans which is characterized by the formation of a clear zone around the disc, this is shown in the results of phytochemical screening of secondary metabolite compounds contained in the ethanol extract of thorn eggplant (Solanum carolinense)

2. The optimal concentration results from the antibacterial activity test showed that the ethanol extract of thorn eggplant fruit at the diameter of the inhibition zone was more effective against Streptococcus mutans bacteria, namely at a concentration of 80% as indicated by the appearance of an inhibition zone diameter of 11 mm (strong).

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