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TESTING THE INHIBITION OF SAWO PUTIK EXTRACT AGAINST ESCHERIA COLI (E-COLI) BACTERIA

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Abstract

Sapodilla, also known as Manilkara zapota or sawo, is being studied as a potential natural remedy for diarrhea caused by Escherichia coli bacteria. The objective of this research is to investigate the inhibitory effects of sapodilla leaf extract on the growth of Escherichia coli bacteria. The method involves formulating sapodilla leaf extract at various concentrations and dividing them into seven groups, including positive and negative controls. The concentrations tested are 2%, 20%, 25%, 30%, and 50% sapodilla leaf extract. The results indicate that sapodilla leaf extract exhibits a mild inhibitory effect on bacterial growth, measuring 6.9 mm in the inhibition zone. Notably, the 50% concentration of sapodilla leaf extract demonstrates a moderate inhibitory effect, with a larger inhibition zone measuring 29.1 mm. This study sheds light on the potential of sapodilla leaf extract as a natural antimicrobial agent, providing valuable insights for further exploration and development of alternative treatments for Escherichia coli-induced diarrhea.

Keywords: *Inhibitory Power, Sapodilla Leaves, Escherichia Coli*

1. INTRODUCTION

Indonesia is known to have a wide variety of plants. The background of Indonesia's archipelago structure has the opportunity for medicinal plants to grow. In fertile soil, they can grow optimally so that they can produce chemical compounds and active ingredients that are used with the best quality in the world. Medicinal plants are used in the form, dried and preserved which have been tested through the extract process with standardized manufacturing (Jawetz, Melnick, 2013). All types of medicinal plants in Indonesia are used to improve welfare and cure various diseases (Hakim, 2022).

The Sapodilla plant or commonly known as sawo fruit (*Manilkara zapota* L) is a fruit plant of the *Sapotaceace* family originating from Central America and Mexico. Sawo leaves contain active compounds that can inhibit and kill bacteria such as Shigella, *Salmonella thypii*, and *Escherchia coli* (*E. coli*). Active substances found in sawo leaves include saponins, tannins, and flavonoids that can inhibit bacterial growth. Saponins inhibit bacterial growth by inhibiting protein synthesis and lowering cell surface tension resulting in leakage. tannins work by lysing the bacterial cell wall while flavonoids work by causing protein cells to clump (Mufti et al., 2017). Diarrhea is a disease caused by microorganisms through food contaminated with bacteria, viruses, protozoa, and parasites. Diarrhea can occur in toddlers, children and adults. Diarrhea causes the death of one in ten children, the number of deaths is 800,000 children every year with 238 cases (Anggreli et al., 2015).

Indonesia experienced fluctuations in the number of diarrhea cases and deaths from 2013 to 2017. In 2013, there were 633 cases with 7 deaths, while in 2014, the numbers increased to 2,549 cases and 29 deaths. The following year, 2015, saw 1,213 cases and 30 deaths, with a higher case fatality rate of 2.47%. In 2016, the numbers decreased significantly to 198 cases and 6 deaths, but the case fatality rate rose to 3.03%. Finally, in 2017, there were 1,725 cases with 34 deaths, and the case fatality rate was 1.97%. The outbreaks were spread across various provinces each year, indicating the importance of continued monitoring and prevention efforts (Sutarjo, 2017). The Ministry of Health's 2018 data shows that the prevalence rate of diarrhea diagnosed by health workers in 2013 was 4.5%. In 2018, there was a 6.8% increase in the number of diarrhea cases. Furthermore, the prevalence rate of diarrhea based on health workers' diagnosis and symptoms in 2013 was 2.4%, with a significant increase of 71.0% in 2018. East Java is the second highest province with 151,878 reported cases of diarrhea, representing a prevalence of 2.6%. Surabaya alone has treated 78,468 cases of diarrhea, which is almost half of the total cases in East Java. It is important to note that one of the contributing factors to diarrhea is bacterial infection (Adhyansyah, 2019). *Escherichia coli* bacteria, which are commonly found in the human digestive tract, can turn pathogenic under certain conditions. This can occur when the amount of Escherichia coli in the digestive tract increases due to factors such as consuming contaminated water or food. Individuals with weakened immune systems, such as infants, the elderly, and those who are ill, are particularly susceptible to pathogenic strains of Escherichia coli. Inhibiting the growth of pathogenic or toxigenic groups like EPEC and ETEC is crucial in preventing infections (Mufti et al., 2017).

According to research by Hasyim et al, (2018) sawo leaf extract with the infuse method can inhibit the growth of Escherichia coli bacteria with the diameter of the inhibition zone produced at a concentration of 5% of 9.72 mm, 10% of 10.68 mm, and 15% of 12.38 mm. From this study it can be concluded that sawo leaves are able to inhibit the growth of Escherichia coli bacteria with the infuse extract method (Kiswandono, 2011). Based on the description above, it is deemed necessary to conduct research to determine the inhibition of sawo leaf extract as an antibacterial Escherichia coli using various concentrations. According to the theory of Sekirov et al (2010), this emphasizes the importance of diversity and balance of gut microbiota (gut flora) in maintaining human health. A healthy gut microbiota has a role in fighting infection with pathogenic bacteria such as Escherichia coli. The use of antimicrobial agents, such as sawo leaf extract, in high doses can disrupt the balance of gut microbiota. This imbalance can lead to bacterial resistance to antibiotics.

Ahmad & Beg (2001) investigated the antibacterial activity of leaf extracts of kaki tree (Diospyros kaki Thunb) and sawo tree (Manilkara zapota L) against Escherichia coli (Sudjadi & Laila, 2006). The results of this study may provide additional insight into the effectiveness of sapodilla tree leaf extracts in inhibiting the growth of E coli (Islam et al., 2013). Meanwhile, other study included testing the antimicrobial activity of Diospyros lotus L. Leaf plant extracts against food-associated pathogenic bacteria, including Escherichia coli (Retnaningsih et al., 2019). The results of this study may provide additional understanding of the potential of this plant leaf extract as an antimicrobial agent.

Setiawati (2018) explored the antibacterial effect of sapodilla tree (Manilkara zapota) leaf extract against Streptococcus mutans, which is a bacterium associated with tooth decay (Prihardini & Wiyono, 2017). Although the bacteria tested were different (Streptococcus mutans), the results of this study may provide insight into the potential of sapodilla leaf extract in inhibiting the growth of pathogenic bacteria.

Interactions Between Microorganisms: The GEB phenomenon often occurs in complex microbial communities, where bacteria that produce antimicrobial compounds may play a role in altering the surrounding environment to the advantage of other bacteria (Taufik & Promosiana, 2015). In this case, sawo leaf extract may play a role (Murwani, 2015). This theory states that sawo fruits might promote the growth of Salmonella Typhi or have other effects that favor the development of these bacteria (Hendro, 2013). Research supporting this theory would highlight possible positive interactions or advantages of other bacteria. In this case, sawo leaf extract may play a role in altering the environment favorable to E coli growth.

Interaction Studies: The study may need to consider the possibility of a gab phenomenon in the experiments. This could involve monitoring the growth of E. coli in the presence of the sawo leaf extract and analyzing the impact of the extract on the growth of the bacteria. In this study it is also important to consider the complex interactions between the sawo leaf extract and E. coli, including possible positive or negative effects on bacterial growth. a term that refers to the increased growth of certain bacteria induced by antimicrobial compounds produced by other bacteria (Octaviani & Syafrina, 2018). In the context of the study, "Inhibition Test of Sawo Leaf Extract Against Escherichia coli Bacteria," several relevant phenomena related to Escherichia coli (E. coli) and sawo leaf extract were encountered: Growth Enhancement Potential: Sawo leaf extract may contain compounds that affect the growth of E. coli. Some of these compounds may have antimicrobial properties, but it is possible that some other compounds in the extract may have a positive impact on E. coli growth, such as facilitating the growth of this microorganism (Isnawati et al., 2018).

Interactions Between Microorganisms: The gab phenomenon often occurs in complex microbial communities, where bacteria that produce antimicrobial compounds may play a role in altering the surrounding environment to the advantage of other bacteria (Paat et al., 2020). In this case, sawo leaf extract may play a role in altering the environment in favor of E. coli growth. Interaction Studies: This could involve monitoring the growth of E. coli in the presence of the sawo leaf extract and analyzing the impact of the extract on the growth of the bacteria. If there is an unexpected increase in growth, this could be a GAB phenomenon that needs to be investigated further. so that the gap between the phenomenon and the existing theory is drawn. The problem is not recommended to be presented in the form of a research question sentence but in the form of a research statement sentence.

2. RESEARCH METHODS

This study is a true experimental post test study using the disc diffusion method to see the effectiveness of manila sawo leaf extract (*Achras zapota* L) in suppressing the growth of E. coli bacteria on diffuse agar medium. The agar diffusion method is the

principle of antibiotics distributed into the media. The antibiotic disk is placed on the surface of the media that has been inoculated pertain, incubated and observed the formation of the zone of inhibition. Effectiveness of antibiotics on the nature of microorganisms.

2.1. Research Tools and Materials

The research employed a variety of tools from the FKUMI laboratory for the extraction of sawo manila. These tools encompassed: (a) petri dish, (b) spirit lamp, (c) mortar and stamper, (d) round ose, (e) hallway term, (f) filter paper, (g) incubator, (h) tweezers, (i) E. coli bacteria, (j) distilled water, and (k) sample. Each of these instruments played a critical role in facilitating the extraction process and subsequent analysis.

2.2. Sawo Leaf Extraction Process

The process of extracting sawo leaves involves a series of consecutive steps. Initially, the leaves are carefully prepared, with young leaves weighing around 1 kg and older leaves weighing 1/5 kg. Next, the leaves are thoroughly washed to ensure cleanliness. They are then dried in a shaded area until they reach the desired dryness, which is determined by their ability to be crushed. The crushed leaves are further processed into a powder called simplia using a blender. To start the extraction process, 50 grams of the sawo leaf powder is weighed and soaked in a 96% ethanol solution for 5 days in a sealed environment to prevent evaporation. After soaking, the mixture is filtered to remove any solid residues. The filtrate is then evaporated on a hotplate until it turns magenta, indicating the concentration of the extract. Finally, the extract is diluted with distilled water according to a specific ratio, determined by a formula below, to achieve the desired concentration for further analysis or applications.

$$M\ 1x\ V1 = M2\ x\ V2$$

Description:

M1 = Concentration of sawo leaf maceration results that will be diluted, namely: 100V1 = Volume of sawo leaf maceration results to be diluted from a concentration of 100%

M2 = concentration to be created

M2 = The volume to be made is 1ml

The concentrations used in this study were 2%, 20%, 25%, 30%, 40%.

Tabel 1. Concentrations Table

No	Concentration (%)	Result of Maceration of Sawo Leaves (Manilkara Zapota)	Distilled Water 9 (Ml)
1	2%	20	980
2	20%	200	800
3	25%	250	750
4	30%	300	700
5	50%	500	500

a. Preparation of Mueller Hinton Agar (MHA) Media

The process of preparing Mueller Hinton Agar (MHA) media includes a series of steps to guarantee proper formulation and sterilization. First, weigh around 3 grams of MHA media and dissolve it in 100 mL of distilled water in a glass beaker. Next, homogenize the solution thoroughly for a uniform mixture. Heat the solution on a hot plate and stir continuously until it boils. Check the pH and add 100 mL of distilled water if needed to reach a pH of 7.4. Let the solution cool, transfer it to an Erlenmeyer flask, cover it with cotton to prevent contamination. Sterilize the media in an autoclave at 121°C for 15 minutes to eliminate any microbial contaminants. After sterilization, cool the media to room temperature and store it in a refrigerator.

b. NB Media Making

To prepare Nutrient Broth (NB) media, first weigh out 0.04 grams of the media. Then, add 5 mL of distilled water to the weighed media.

c. Making Paper Disks

To craft paper disks, begin by preparing disc paper. Then, cut the paper into pieces with a 6 mm diameter. Next, heat the cut paper in an oven for 1 hour at 180°C.

d. Preparation of Bacterial Suspension

To prepare a bacterial suspension, first, obtain pure Escherichia coli bacteria. Then, using a round loop, transfer the bacteria into Nutrient Broth (NB) media and homogenize thoroughly. Incubate the mixture for 24 hours. Following the incubation period, take 1 loopful of the resulting culture and transfer it into 1 mL of saline solution (NaCl). Homogenize the solution to ensure thorough mixing.

e. Inhibitory Test Procedure of Sawo Leaf (*Manilkara zapota*)

The procedure for conducting the inhibitory test of Sawo leaf (*Manilkara zapota*) involves several sequential steps aimed at evaluating its antimicrobial properties. Firstly, prepare 10 sterile petri dishes. Next, spread Mueller Hinton Agar (MHA) media evenly on a hot plate. Subsequently, dilute the sawo leaf extract to predetermined concentrations of 2%, 20%, 25%, 30%, and 50%. Utilize chloramphenicol antibiotics for the positive control and distilled water for the negative control, soaking disc paper for 15 minutes. Then, pour 10 mL of media into each Petri dish and allow it to solidify. Add 1 mL of bacterial suspension onto the solidified media and homogenize. Using a sterile cotton swab dipped into a test tube containing bacterial suspension, evenly scrape the bacteria onto the media. Allow 5-10 minutes for bacterial diffusion within the media. Subsequently, immerse disc papers in sawo leaf extract at each predetermined concentration and in the positive control with chloramphenicol antibiotic for 15 minutes. Apply the soaked disc papers onto the MHA media using sterile tweezers according to the predetermined concentrations of sawo leaf extract. Incubate the Petri dishes for 24 hours at 37°C. Finally, observe and measure the zones of inhibition around the disc papers, indicating the effectiveness of the sawo leaf extract against bacterial growth.

3. RESULTS AND DISCUSSION

3.1. Research Result

The findings of the investigation on the Zone of Inhibition caused by sawo leaf extract (Manilkara zapota) against Escherichia coli bacteria are as follows:

Table 2. Type Inspection and Result of Sawo Leaf Extract Concentration

No	Type of Inspection	Result						
		Sawo Leaf Extract Concentration						
1	Inhibition of Sawo Leaf Extract Against Escherichia coli	2%	20%	25%	30%	50%	Control (+)	Control (-)
		6.9 mm	8.1 mm	8.2 mm	9.2 mm	9.5 mm	29.1 mm	0

3.2. Discussion

The findings from the research carried out in the Microchemistry laboratory and the Unsyiah Kesmavet laboratory revealed the results of the inhibition test of sawo leaf extract (*Manilkara zapota*) against *E.coli* bacteria. The experiment involved 5 different concentrations - 2%, 20%, 25%, 30%, and 50%. The observations were made within a 24-hour period, with a positive control using aquadest. Notably, the 2% concentration showed an inhibition zone of 6.9 mm within 24 hours, falling under the weak category of <10 mm (Mulyadi et al., 2017).

Concentration of 20% in the incubation period for 1x24 hours formed an inhibition zone of 8.1 mm in the area around the paper disk, which means that sawo leaves (*Manilkara zapota*) have the potential as anti-bacterial. The concentration of 25% was carried out during the incubation period for 1x24 hours to form an inhibition zone of 8.2 mm in the area around the paper disk, which means that sawo leaves (*Manilkara zapota*) have potential as anti-bacterial. This inhibition zone is included in the weak category of <10mm (Mulyadi et al., 2017). 30% concentration was carried out during the incubation period for 1x24 hours to form an inhibition zone of 9.2 mm in the area around the paper disk which means that the leaves of sawo (*Manilkara zapota*) have potential as anti-bacterial. This inhibition zone is included in the weak category of <10mm (Mulyadi et al., 2017).

The 50% concentration formed an inhibition zone of 9.5 mm in 24 hours in the area around the paper disk, which means that sawo leaves are effective in inhibiting the growth of *e.coli* bacteria with a ratio between extracts and solvents, namely 400 micro of sawo leaf extract (*Manilkara zapota*) and 600 micro of aquadest. This inhibition zone is included in the medium category of 10-15 mm (Mulyadi et al., 2017).

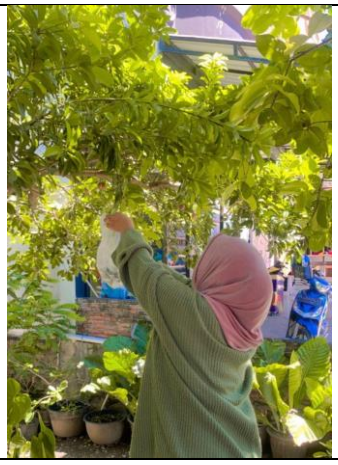
The sawo leaf (*Manilkara zapota*) extract showed a 6.9 mm inhibition zone at 2% concentration, while the largest inhibition of 9.5 mm was observed at 50% concentration. As the concentration rises, the diameter of the inhibition zone also tends to increase. The effectiveness of antibacterial substances is directly affected by the concentration level. Higher concentrations result in a greater amount of antibacterial active compounds, enhancing their ability to eliminate bacteria (Mufti et al., 2017).

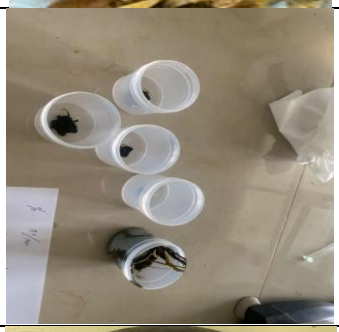
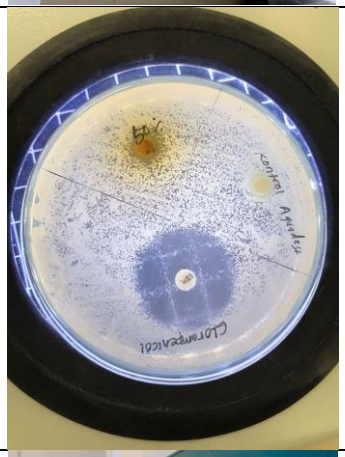
It Therefore, it is evident that the leaves of the sawo tree (Manilkara zapota) possess the ability to hinder the development of Escherichia coli bacteria. This demonstrates that sawo leaves (Manilkara zapota) contain antibacterial properties such as saponins, tannins, alkaloids, and flavonoids, which impede bacterial growth. This observation aligns with previous theories. Saponins function by reducing the tension of the bacterial cell wall, leading to instability in the cell membrane. Consequently, the growth of enzymes crucial for bacterial survival is inhibited. As a result of decreased cell wall surface tension, leakage occurs, causing the release of intracellular compounds. This, in turn, hampers the growth of bacterial cells (Mulft, Bahar, and Arisanti, 2017).

The maration method was utilized in this study to extract the active substances from sawo leaves (manilkara zapota) using 96% ethanol as a solvent. 96% ethanol is an effective solvent with both polar and non-polar properties, allowing for the perfect extraction of the active substances in sawo leaves (manilkara zapota). The researchers found that as the concentration of the solvent increased, the diameter of the inhibition zone became more visible. This is attributed to the presence of active substances such as saponins, flavonoids, tannins, and alkaloids in sawo leaves (manilkara zapota). Saponins act as antibacterial agents by inhibiting the growth of enzymes that are essential for bacterial survival. This leads to a decrease in cell wall surface tension and the leakage of intra-cellular compounds, ultimately inhibiting the growth of bacterial cells (Mufti et al., 2017).

Flavoid has a high content of compounds in plants that inhibit growth by causing damage to the permeability of bacterial cell walls, chromosomes, and lysosomes as a result of interaction between flavonoids and bacterial DNA (Yunika, 2017). Tannins work as antibacterials by inhibiting the formation of bacterial cell wall polypeptides that cause the lysis of the bacterial cell wall. Tannins also have a spasmolytic effect that can reduce intestinal peristalsis and wrinkle the bacterial cell wall, causing disruption of bacterial cell permeability, so that the cell wall layer is not formed completely and causes cell death (Marfuah et al., 2018).

Table 3. Research Process Picture

Research Process Picture	Explanation
	Samples were taken next to the residents' houses where 1.5 kg of young sawo leaves and 1 kg of old leaves were taken.

Research Process Picture	Explanation
	Followed by the drying process in the room until completely dry, not exposed to direct sunlight.
	The process of mixing extracts with distilled water
	Putting lendis that has been soaked in extracts into Petri dishes to see the concentration of extract inhibition against *e.coli* bacteria.
	Adding *e.coli* bacteria to see how much inhibition of *e.coli* bacteria farom sawo leaf extract.

4. CONCLUSION

After analyzing the findings from the study, it is evident that the sawo leaves extract (*Manilkara zapota*) has the ability to hinder the growth of Escherichia coli bacteria. At a concentration of 2%, a 6.9 mm inhibition zone diameter was observed, falling under the weak category. Similarly, at a concentration of 20%, an 8.1 mm inhibition zone diameter was recorded, also categorized as weak.

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