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Testing The Antimicrobial Effectiveness of Manila Sauce Leaves (Manilkara Zapota L) against Bacteria Escherichia Coli: In Vitro Study

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Abstract

Diarrhea remains a significant public health concern globally, particularly affecting children under five years old. Traditional herbal remedies, including Manila Sapodilla (Manilkara zapota L) leaves, have been utilized in Indonesia for treating diarrhea due to their perceived efficacy and safety. This in vitro study aimed to assess the antimicrobial effectiveness of Manila Sapodilla leaf extract against Escherichia coli bacteria, a common cause of diarrhea. True experimental design was employed, utilizing various concentrations (25%, 50%, 75%, and 100%) of Manila Sapodilla leaf extract. The extract was obtained through maceration using ethanol followed by dilution with distilled water. Antimicrobial activity was evaluated using the Kirby-Bauer diffusion method, measuring inhibition zones formed around Escherichia coli colonies. Results demonstrated that Manila Sapodilla leaf extract exhibited inhibitory effects against Escherichia coli at all tested concentrations, albeit with varying degrees of effectiveness. The inhibition zones ranged from 5.37 mm to 8.53 mm, indicating resistance. Positive control using Ciprofloxacin displayed a significantly larger inhibition zone (21.75 mm), indicating sensitivity. These findings suggest the potential of Manila Sapodilla leaf extract as an alternative treatment for Escherichia coli infections. However, further research, including phytochemical analysis and exploration of different extraction methods and concentrations, is warranted to elucidate its therapeutic potential fully. This study contributes to the ongoing search for alternative herbal-based antibiotics to combat bacterial infections effectively.

Introduction

Diarrhea is a contagious disease and is characterized by symptoms such as changes in the shape and consistency of the stool from becoming soft to liquefying and an increase in the frequency of defecation more than usual accompanied by vomiting, thus causing sufferers to experience a lack of fluids in the body or dehydration which in In the end, if you don't get help immediately it can cause seriousness and even death. Diarrhea still ranks third as the leading cause of death for children under five years old. According to data from the World Health Organization (WHO), diarrhea is the main cause of death in children under 5 years of age throughout the world. WHO also reports that every year there are around 1.7 billion cases of diarrhea in the world, with approx 525,000 deaths caused by diarrhea in children.1,2,3 In Indonesia, the Ministry of Health (Kemenkes) also notes that diarrhea is one of the diseases that occurs frequently and is a public health problem.

According to a household health survey conducted by the Ministry of Health in 2018, diarrhea is the disease most frequently experienced by the Indonesian population (Megatsari et al., 2021). Indonesia has a variety of cultures and regional cultures. There is a legacy passed down

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from generation to generation that still provides treatment for various diseases traditionally from various medicinal plants found in the surrounding environment. Indonesian people tend to rely on traditional concoctions made from natural ingredients, including leaves, as a way to treat diarrhea (Budiarti et al., 2020; Hernawati et al., 2022). This may be due to the belief in the safety and effectiveness of traditional herbal medicines.4,5,6 One plant that has the potential as an anti-bacterial agent to treat diarrhea is Manila sapodilla leaves (Manilkara zapota). Manila sapodilla leaves have long been used in traditional medicine in several regions in Indonesia (Setiani et al., 2022). Several scientific studies have shown that Manila sapodilla leaves contain active compounds that have antimicrobial properties and can fight the growth of bacteria such as Escherichia coli, including bacteria that cause diarrhea.

By utilizing the potential of Manila sapodilla leaves as a natural ingredient in treating diarrhea, it is hoped that it can provide an effective and safe alternative treatment for the Indonesian people (Akhtar, 2022). However, further research needs to be carried out to understand in more depth the mechanism of action of Manila sapodilla leaves, the possible side effects, and their effectiveness in treating diarrhea in humans.7,8,9 Previously, a study was carried out entitled "Inhibitory Test of Sapodilla Leaf Extract against Escherichia coli Bacteria in Vitro" with concentrations of 15%, 30%, 45%, 60% and 100%. The results of this research state that manila sapodilla leaf extract at a concentration of 100% is categorized as very strong in inhibiting bacteria E. coli. So far, the concentration has been high, there has been no research with a concentration < 100% at the Faculty of Medicine, Indonesian Muslim University. Therefore, it is necessary to study "Testing the Antimicrobial Effectiveness of Manila Sapodilla Leaves (Manilkara Zapota L) Against Escherichia Coli Bacteria: In Vitro Study". Where the author will use concentrations of 25%, 50%, 75% and 100%.

Based on the background above, a problem formulation can be formulated, namely: What is the level of effectiveness of manila sapodilla leaf extract (Manilkara zapota L.) against Escherichia coli bacteria? Research Objectives To determine the effectiveness of sapodilla leaf extract (Manilkara zapota L.) against Escherichia coli bacteria.

Methods

The research used in this research is a true experimental design. In this design the researcher can control all variables that influence the course of the experiment. In this research, researchers used a maceration method using ethanol and dilution with distilled water to see the effectiveness of Manila Sapodilla Leaf Extract (Manilkara zapota L.) as an antimicrobial against Escherichia coli bacteria by measuring the inhibition zone formed. The research was conducted at the Research Laboratory of the Faculty of Medicine, Indonesian Muslim University, Jl. Urip Sumohardjo KM 5, Makassar City. The samples used in this research were Manila Sapodilla Leaves (Manilkara zapota L.) without previous extraction treatment and then extracted in concentrations of 25%, 50%, 75% and 100%. The tools used in this research consisted of masks, handscoons, petri dishes, ovens, glass jars, blenders, tube racks, test tubes, measuring cups, stir sticks, Erlenmeyer flasks, spirit lamps, round tubes, analytical scales, vernier calipers, blank disc, rotary evaporator, filter paper, autoclave, water bath, incubator, aluminum foil, filter, tweezers, 1 ml and 10 ml volume pipettes, mortar and stamper.

Results and Discussion

This research was conducted in the Research Laboratory of the Faculty of Medicine, Indonesian Muslim University in September 2023. This research was conducted with the aim of determining the antibacterial effectiveness of manila sapodilla leaf extract (Manilkara zapota) on the growth of Escherichia coli. Before the antibacterial test, an extraction process was

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carried out using 96% ethanol solvent to obtain manila sapodilla leaf extract. The manila sapodilla leaf extract solution which has been macerated using ethanol solvent is then evaporated to produce a concentrated extract with a paste-like texture and a dark green color. Then the antibacterial activity test was carried out in vitro against Escherichia coli bacteria using the Kriby-Bauer diffusion method. The extract's ability to produce antibacterial compounds is indicated by the formation of an inhibitory zone in the area around the Escherichia coli bacteria. The results showed that manila sapodilla leaf extract with varying concentrations of 25%, 50%, 75% and 100% produced an inhibitory zone in the area around the Escherichia coli bacteria with the interpretation of resistance. The results of calculating the inhibition zones formed at various concentrations of sapodilla leaf extract, positive control and negative control can be seen in table 1.

Table 1. Results of Observations on the Effectiveness of Manila Sapodilla Leaves (Manilkara zapota) against Escherichia coli

Research Materials	**Concentration**	**Zone of Inhibition in Escherichia coli (mm)**	**Interpretation of Growth Barrier Responses**
Leaf Extract Hello Manila (Manilkara zapota L.)	25%	5,37	Resist
	50%	8,11	Resist
	75%	8,53	Resist
	100%	8,27	Resist
Positive Control (+)	Ciprofloxacin	21,75	Sensitive
Negative Control (-)	Aquadest	0	Resist

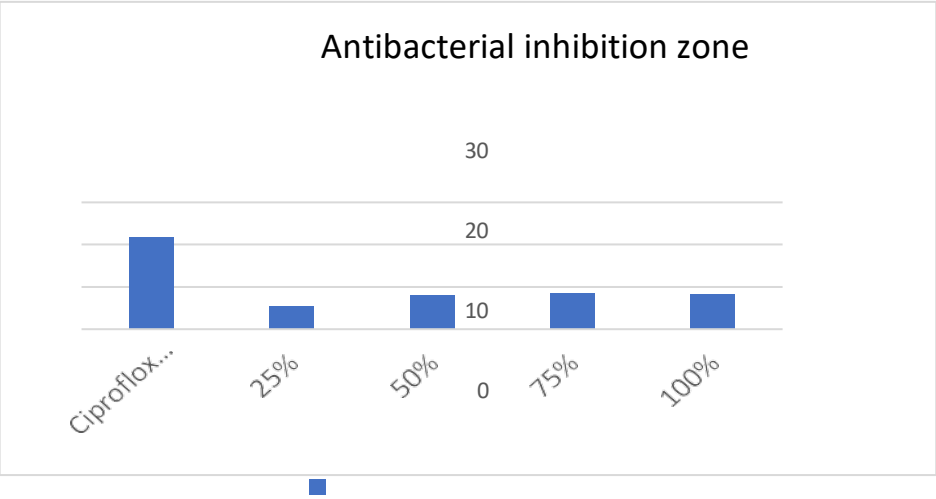

Figure 1. Comparison graph of the inhibition zone for Escherichia coli bacteria

Based on the data in table 4.1, the results showed that manila sapodilla leaf extract (Manilkara zapota L.) at a concentration of 25% formed an inhibition zone of 5.37 mm where ≤ 14 mm with the interpretation of resistance, at a concentration of 50% formed an inhibition zone of 8.11 mm where ≤ 14 mm with a resistant interpretation, a concentration of 75% forms an inhibition zone of 8.53 mm where ≤ 14 mm with a resistant interpretation and a concentration of 100% forms an inhibition zone of 8.27 mm where ≤ 14 mm with a resistant interpretation. In the positive control, namely the antibiotic Ciprofloxacin, an inhibition zone of 21.75 mm was formed, which is ≥ 20 mm, with the interpretation being sensitive to the growth of Escherichia coli, whereas in the negative control using distilled water, no inhibition zone was formed. The following is the attachment for the results of the effectiveness test treatment that has been carried out:

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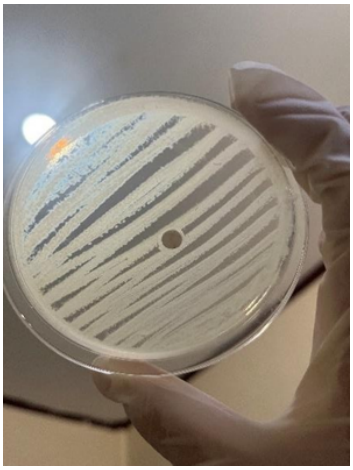

Figure 2. Negative control with distilled water shows that there is no inhibition zone formed against Escherichia Coli bacteria

Figure 3. Antimicrobial Manila Sapodilla Leaf Extract (Manilkara zapota L) concentration of 25% forms an inhibitory zone with a diameter of 5.37 mm with an interpretation of resistance to Escherichia Coli

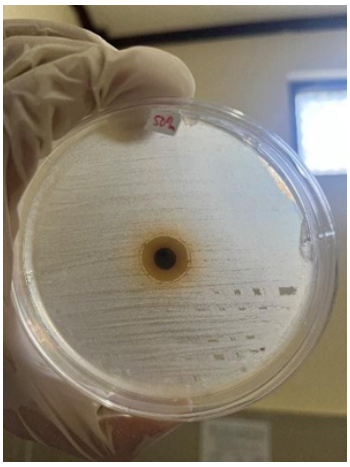

Figure 4. Antimicrobial Manila Sapodilla Leaf Extract (Manilkara zapota L) concentration of 50% forms an inhibitory zone with a diameter of 8.11 mm with an interpretation of resistance to Escherichia Coli

ISSN 2721-1304 (Print), ISSN 2721-1207 (online)

Figure 5. Antimicrobial Manila Sapodilla Leaf Extract (Manilkara zapota L) concentration of 75% forms an inhibitory zone with a diameter of 8.53 mm with an interpretation of resistance to Escherichia Coli

Figure 6. Antimicrobial Manila Sapodilla Leaf Extract (Manilkara zapota L) concentration of 100% forms an inhibitory zone with a diameter of 8.27 mm with an interpretation of resistance to Escherichia Coli

Figure 7. The antibiotic Ciprofloxacin forms an inhibitory zone with a diameter of 21.75 mm with a sensitive interpretation for Escherichia Coli bacteria

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This research uses True experimental design research to determine the existence of an inhibitory zone formed in manila sapodilla leaf extract (Manilkara zapota L). Manila sapodilla leaves contain chemical compounds in the form of flavonoids, saponins, tannins and alkaloids in manila sapodilla extract (Manilkara zapota L.). These contents have antibacterial activity which can inhibit bacterial growth. In terms of the mechanism of action of each compound, flavonoids are the largest group of phenols in plants, these compounds can easily dissolve in water and polar solvents. Flavonoids are known as antioxidants that have antibacterial activity. The mechanism of antibacterial action of phenolic compounds is by causing coagulation or protein clumping which causes denaturation of cells so that the protein no longer functions. Saponin is able to inhibit bacterial growth by inhibiting protein synthesis and reducing the surface tension of bacterial cells, resulting in leakage. The mechanism of action of saponin compounds is to form complex compounds with cell membranes through hydrogen bonds, so that the permeability properties of cell walls can be damaged, resulting in bacterial cell death. 31,37 The active compounds in manila sapodilla leaf extract can be said to inhibit the growth of Escherichia coli bacteria if an inhibitory zone forms around them. Sodium Agar media growing colonies of Escherichia coli bacteria with a large inhibitory zone diameter that corresponds to the inhibitory zone classification. The positive control in the study showed a significant difference between the negative control and the concentration of sapodilla leaf extract, where the inhibition zone formed in the positive control showed the largest inhibition zone, namely 21.75 mm, which means the positive control used was ≥ 21 mm with a sensitive interpretation for the growth of E coli.

The positive control in this study used the antibiotic Ciprofloxacin. Ciprofloxacin is a bactericide from the fluoroquinolone drug class with a working mechanism of inhibiting DNA replication by inhibiting bacterial DNA topoisomerase and DNA-gyrase. Of the fluoroquinolone class, ciprofloxacin is the most effective against gram-negative bacilli (especially Enterobacteriaceae such as Escherichia coli, Salmonella spp., Shigella spp., and Neisseria). Ciprofloxacin also has effectiveness against several gram-positive bacteria. 38,37 Research conducted by Hermawati (2023) regarding the sensitivity test of ciprofloxacin against Escherichia coli in vitro showed that the inhibition zone was > 21 mm which was included in the sensitive category. So this supports researchers using the antibiotic ciprofloxacin as a positive control in this study.39 In this study, regarding the effectiveness of sapodilla leaf extract (Manilkara zapota L.) against Escherichia coli bacteria, the results were that sapodilla leaf extract at concentrations of 25%, 50%, 75% and 100% showed an inhibition zone on Natrium Agar medium that had been grown. E. Coli which is ≤ 14 mm with the interpretation of resistant. This research is in line with Hasanah (2018) and Tampubolon et al. (2023) that it has not been able to inhibit the growth of E. coli bacteria. 8,40 However, research from Primadiamanti (2020) found that it could inhibit E. coli bacteria even though the results were intermediate. or the ability to inhibit it was moderate in the test bacteria. 41 And in the research of Mufti (2017) and Sembiring (2022), the results of sapodilla leaf extract were categorized as sensitive to the growth of E. coli bacteria. 7,42

This research uses True experimental design research to determine the existence of an inhibitory zone formed in manila sapodilla leaf extract (Manilkara zapota L) (Mohd et al., 2022). Manila sapodilla leaves contain chemical compounds in the form of flavonoids, saponins, tannins and alkaloids in manila sapodilla extract (Manilkara zapota L.) (Bangar et al., 2022). These contents have antibacterial activity which can inhibit bacterial growth. In terms of the mechanism of action of each compound, flavonoids are the largest group of phenols in plants, these compounds can easily dissolve in water and polar solvents. Flavonoids are known as antioxidants that have antibacterial activity. The mechanism of antibacterial

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Conclusion

The inhibition zone for the growth of Escherichia coli bacteria given manila sapodilla leaf extract (Manilkara zapota L.) with a concentration of 25% was 5.37 mm (resistant). The inhibition zone for the growth of Escherichia coli bacteria given manila sapodilla leaf extract (Manilkara zapota L.) with a concentration of 50% was 8.11 mm (resistant). The inhibition zone for the growth of Escherichia coli bacteria, which was given manila sapodilla leaf extract (Manilkara zapota L.) with a concentration of 75% was 8.53 mm (resistant). The inhibition zone for the growth of Escherichia coli bacteria given manila sapodilla leaf extract (Manilkara zapota L.) with a concentration of 100% was 8.27 mm (resistant). There is no zone of inhibition for the growth of Escherichia coli bacteria given distilled water (negative control) was 0 mm (resistant). The inhibition zone for the growth of Escherichia coli bacteria is given ciprofloxacin (positive control) was 21.75 mm (sensitive).

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Suggestion

Before conducting research, it is recommended to first carry out a phytochemical test on the sapodilla leaf extract to ensure the active substance content in the sapodilla leaf extract. For future researchers, it can be used as a reference for further research on manila sapodilla leaf extract (Manilkara zapota L) using different solvents, varying different concentrations and using other parts of the sapodilla plant. Develop research on alternative medicines for the treatment of diseases caused by Escherichia coli bacteria as the discovery of herbal-based antibiotics.

References

Akhtar, M. A. (2022). Anti-inflammatory medicinal plants of Bangladesh—a pharmacological evaluation. *Frontiers in pharmacology*, *13*, 809324. https://doi.org/10.3389/fphar.2022.809324

Bangar, S. P., Sharma, N., Kaur, H., Kaur, M., Sandhu, K. S., Maqsood, S., & Ozogul, F. (2022). A review of sapodilla (Manilkara zapota) in human nutrition, health, and industrial applications. *Trends in Food Science & Technology*, *127*, 319-334. https://doi.org/10.3389/fpls.2022.999270

Budiarti, M., Maruzy, A., Mujahid, R., Sari, A. N., Jokopriyambodo, W., Widayat, T., & Wahyono, S. (2020). The use of antimalarial plants as traditional treatment in Papua Island, Indonesia. *Heliyon*, *6*(12).

Hasanah N, Harso Kardhinata E, Nasution J. *Uji Anti Bakteri Ekstrak Daun Sawo Manila (Manilkara Zapota) Terhadap Escherichia Coli Antibacterial Test of Sapilla Manila (Manilkara Zapota) Leaf Extract Against Escherichia Coli*. Vol 1.; 2018. http://jurnalmahasiswa.uma.ac.id/index.php/jibioma

Hermawati, A. H., Surtini, S., & Hariyanto, H. (2023). Uji Antibiotik Ciprofloxacin Terhadap Pertumbuhan Escherichia Coli Secara In Vitro. *Jurnal Insan Cendekia*, *10*(3), 181-188. https://doi.org/10.35874/jic.v10i3.1187

Hernawati, D., Putra, R. R., & Meylani, V. (2022). Indigenous vegetables consumed as lalapan by a Sundanese ethnic group in West Java, Indonesia: Potential, traditions, local knowledge, and it's future. *South African Journal of Botany*, *151*, 133-145. https://doi.org/10.1016/j.sajb.2022.09.007

Megatsari, H., Laksono, A. D., & Moelyaningrum, A. D. (2021). Diarrhea Prevalence in East Java, Indonesia: Does Access to Sanitation and Health Behavior Ecologically Related?. *Indian Journal of Forensic Medicine & Toxicology*, *15*(4), 1276-1283.

Millanao, A. R., Mora, A. Y., Villagra, N. A., Bucarey, S. A., & Hidalgo, A. A. (2021). Biological effects of quinolones: a family of broad-spectrum antimicrobial agents. *Molecules*, *26*(23), 7153. https://doi.org/10.3390/molecules26237153

Mohd Israfi, N. A., Mohd Ali, M. I. A., Manickam, S., Sun, X., Goh, B. H., Tang, S. Y., ... & Chan, K. W. (2022). Essential oils and plant extracts for tropical fruits protection: From farm to table. *Frontiers in Plant Science*, *13*, 999270. https://doi.org/10.3389/fpls.2022.999270

Mufti, N., Bahar, E., & Arisanti, D. (2017). Uji daya hambat ekstrak daun sawo terhadap bakteri Escherichia coli secara in vitro. *Jurnal Kesehatan Andalas*, *6*(2), 289-294. https://doi.org/10.25077/jka.v6.i2.p289-294.2017

ISSN 2721-1304 (Print), ISSN 2721-1207 (online)

Parnomo, T., & Pohan, D. J. (2021). Test the Effectiveness of Aloe Vera Extract on the Growth of Escherichia coli in vitro. *International Journal of Health Sciences and Research*, *11*(8), 211-224.

Primadiamanti, A., Purnama, R. C., & Aulia, R. (2020). Uji Daya Hambat Daun, Kulit Batang Dan Buah Sawo Manila Muda (Manilkarazapota L) Terhadap Bakteri Escherichia Colidan Staphylococcus Aureus Menggunakan Metode Difusi Sumuran. *Jurnal Analis Farmasi*, *5*(2), 135-141. https://doi.org/10.33024/jaf.v5i2.4086

Sembiring, P. (2022). Formulasi Dan Uji Ektivitas Antibakteri Sediaan Lotion Ekstrak Etanol Daun Sawao Terhadap Bakteri Stapylococcuc aureus dan Escherchia coli. *Jurnal Penelitian Farmasi & Herbal*, *4*(2), 20-28. https://doi.org/10.36656/jpfh.v4i2.853

Setiani, S., Setiawan, E., & Huang, W. C. (2022). Taneyan Lanjang shared home gardens and sustainable rural livelihoods of ethnic Madurese in Madura Island, Indonesia. Sustainability, 14(10), 5960. https://doi.org/10.3390/su14105960

Tampubolon, M. I., & Hutabarat, R. E. B. (2023). Uji aktivitas antibakteri ekstrak n-heksana daun sawo (Manilkara zapota L) terhadap pertumbuhan bakteri Staphylococcus aureus dan Escherichia coli. *Journal of Pharmaceutical and Sciences*, 1443-1455. https://doi.org/10.36490/journal-jps.com.v6i4.225

Vartika, Chaudhary, M., Bhagyawant, S. S., & Srivastava, N. (2022). Effects of prednisolone derivative and panaxydol: Biosurfactants on cell wall integrity of acne-causing resistant bacteria. *Cell Biochemistry and Biophysics*, 1-15. https://doi.org/10.1007/s12013-021-01038-0

ISSN 2721-1304 (Print), ISSN 2721-1207 (online)