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INVESTIGATION OF THE ANTIBACTERIAL EFFICACY OF DERSONO WATER APPLE LEAVES (*SYZYGIUM MALACCENSE*) AS A SUBSTITUTE THERAPY FOR *STREPTOCOCCUS PYOGENES* INFECTIONS

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ABSTRACT

Streptococcus pyogenes caused different illnesses, including pharyngitis and dermatological infections. The Dersono water apple leaves (Syzygium malaccense) are recognized for their bioactive ingredients, which may serve as antibacterial agents. This study purpose was to determine the antibacterial potential of ethanol extract and acetone fraction of Syzygium malaccense against Streptococcus pyogenes infection. This research was a laboratory experiment, a post-test only with control group design. Assessing growth inhibition by measuring the diameter of halo around the well of blood agar plate. Statistical tests using the Sapiro-Wilk test, Lavene test, Kruskal Wallis test and Mann-Whitney test. The study's findings demonstrated that the ethanol extract 25% (11.80 mm) and 50% (16.51) concentrations and acetone fraction of Dersono water apple leaves, at 25% (11.25 mm) and 50% (12.08) concentrations, had strong inhibitory power. The concentrations of 6.25% (8.15 mm) and 12.50% (9.63 mm), the ethanol extract's inhibitory power was superior than the concentrations of 6.25% (4.42 mm) and 12.50% (3.38 mm) acetone fraction, had moderate range. Both the ethanol extract and the acetone fraction's phytochemical analyses identified alkaloids, tannins, and saponins. The conclusion was ethanol extract and acetate fraction of Dersono water apple leaves have antibacterial effects against Streptococcus pyogenes.

KEYWORDS:

Syzygium malaccense Leaves, Ethanol Extract, Acetone Fraction Antibacterial Agents, Streptococcus pyogenes

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INTRODUCTION

Streptococcus pyogenes is responsible for more than 500,000 deaths per year worldwide, especially in low- and middle-income countries [1]. *Streptococcus pyogenes* is well adapted to the human host, resulting in asymptomatic infections, bacterial pharyngotonsillitis, pyoderma, severe scarlet fever, poststreptococcal glomerulonephritis, skin infections, severe soft tissue infections, necrotizing fasciitis, bacteremia (sepsis), endocarditis, pneumonia, empyema, and streptococcal toxic shock syndrome. The

infectious potential of these diseases is without age restrictions [2,3].

This bacterium uses various virulence determinants to allow colonization, spread within the host, and transmission, interfering with the innate and adaptive immune responses to infection [2]. *Streptococcus pyogenes* is also known as Group A ß-hemolytic Streptococcus (GAS), which is a Gram-positive bacterium. This bacterium can be easily identified in a microbiology laboratory through its ability to hemolyze blood in culture media. This bacterium is highly virulent due to its enzyme and toxin production, and its ability to hemolyze. The fluctuating global GAS epidemiology is characterized by the emergence of new GAS mutants/clones, with higher virulence. Identification of GAS isolates that are less sensitive to penicillin increases the risk of macrolide resistance as the first line in the treatment of this bacterium [3]. This quite worrying potential for resistance requires action to prevent and control the spread of resistant mutant strains. For most Streptococcus infections in pediatric patients and pregnant women, the use of beta-lactam antibiotics remains the first line of choice, but the use of empirical broad-spectrum antimicrobial therapy increases the spread of Multiple Drug Resistance Streptococcus etiological agents. This fact creates a vicious circle that must be overcome with antibiotic policy [4]. Antibiotic policies include tightening the implementation of antibiotic use regulations involving various cross-sectors from the government, academics, health workers, pharmaceutical industry, and the community. One step to prevent antibiotic resistance in real terms is to find new agents/compounds that can be developed or have the potential as antibiotics derived from natural materials.

Indonesia has a biodiversity that can be utilized as herbal medicine. Herbal medicine has been used empirically to treat various infectious diseases. Herbal products have an important role in drug development, especially as antibiotics. Phytochemical screening shows that *Syzygium malaccense* leaf extract contains flavonoids, tannins, quinones, phenols, and triterpenoids/steroids[5].

Syzygium malaccense leaf extract is known to have antioxidant, antibacterial, repellent/ mortality of Aedes aegepty larvae, cytotoxic, antifungal, antidiabetic,

hepatoprotective, anti-inflammatory, and analgesic activities [5,6,7,8]. Existing research states that *Syzygium malaccense* leaf extract shows antibacterial activity against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Pseudomonas aeruginosa bacteria [9].

METHODS

This study used a laboratory experimental method with Streptococcus pyogenes bacteria. The material test was 96% ethanol extract and the acetone fraction of Dersono water apple leaves. The initial step was a determination test of the Dersono water apple leaves. The first stage of the study involved preparing Dersono water apple leaf extract using a maceration method with 96% ethanol to obtain a thick extract. Then, concentrations of 6.25%, 12.5%, 25%, and 50% were prepared using dimethyl sulfoxide (DMSO) as a negative control. Amoxicillin was used as a positive control. The second stage involved preparing the acetone fraction from the first stage extract to obtain a thick fraction, and concentrations were set at 6.25%, 12.5%, 25%, and 50%. The third stage involved qualitative phytochemical testing of the ethanol extract and the acetone fraction of Dersono water apple (Syzygium malaccense) leaves. The fourth stage involved preparing a Streptococcus pyogenes bacterial culture on blood agar. The fifth stage involved conducting antibacterial tests using the well method on test materials from ethanol extract and the acetone fraction of Dersono water apple leaves. Data were collected in the form of the average diameter of the growth inhibition zone for Streptococcus pyogenes bacteria formed around the wells using the Kirby-Bauer method. The sixth stage involved a qualitative phytochemical test to determine the secondary metabolite content in the ethanol extract and the acetone fraction of Dersono water apple leaves.

Statistical tests used included the Lavene test, the Sapiro-Wilk test, the Kruskal Wallis test, and the Mann-Whitney test. Data from the treatment group were compared with the negative control group to determine the inhibitory effect on bacterial growth, and compared with the positive control group (amoxicillin) to determine the strength of the inhibitory effect.

RESULT AND DISCUSSION

Determination of plants used as research subjects obtained Family: Myrtaceae,

Species: Syzygium malaccense (L) Merr. and L. M. Perry, Synonyms: Eugenia malaccensis L. The maceration process of 1000 grams of Dersono water apple leaf simplicia produced 117.65 grams of thick extract.

Data from the antibacterial test results of ethanol extract of Dersono water apple leaves (*Syzygium malaccense*) against *Streptococcus pyogenes* bacteria can be seen in table 1 below. Antibacterial test data of the acetone fraction of Dersono water apple leaves (*Syzygium malaccense*) against *Streptococcus pyogenes* bacteria obtained an average diameter as shown in Table 2. Based on tables 1 and 2, the use of DMSO negative control was appropriate, because DMSO was unable to inhibit the growth of S. Pyogenes bacteria (mean inhibition zone size = 0 mm). Dimethyl sulfoxide (DMSO), an organosulfur compound, is widely used as the gold standard solvent in biological research[28]. DMSO is used to dissolve a wide variety of natural and synthetic products, including isolated secondary metabolites, plant extracts, modified lead molecules and proteins, in vitro antibiofilm assays[29]. The average diameter of the inhibition zone for the growth of S. Pyogenes bacteria from the antibacterial test of ethanol extract and acetone fraction of Dersono water apple Leaves increased with increasing concentration of ethanol extract and acetone fraction. Increasing the concentration from the smallest to the largest has an impact on the diameter of the inhibition zone formed, because the more effective compounds in the extract or fraction that can inhibit bacterial growth increase[30]. Phytochemical tests of secondary metabolites of the test materials in this study obtained saponins, tannins, and alkaloids, as shown in the table 3.

Table 1. Average Diameter of Inhibition of *Streptococcus pyogenes* Bacterial Growth by Ethanol Extract of Dersono Water Apple Leaves (*Syzygium malaccense*)

Treatment Group	Inhibition zone size (mm)			Mean of Inhibition zone size (mm± SD)
	Repetition I	Repetition II	Repetition III	
Negative control (DMSO)	0	0	0	0
Dersono water apple Leaf Ethanol Extract 6.25%	8.58	7.99	7.89	8.15±0.30
Dersono water apple Leaf Ethanol Extract 12.5%	11.55	8.85	8.5	9.63±1.36
Dersono water apple Leaf Ethanol Extract 25%	12.19	11.52	11.69	11.80±0.28
Dersono water apple Leaf Ethanol Extract 50%	17.62	16.4	15.51	16.51±0.86
Positive control (amoxicillin)	45.86	39.4	39.78	41.68±2.95

Table 2. Average Diameter of inhibition of Growth of *Streptococcus pyogenes* Bacteria by Acetone Fraction of Dersono Water Apple Leaves (*Syzygium malaccense*)

Treatment Group	Inhibition zone size (mm)			Mean of Inhibition zone size (mm± SD)
	Repetition I	Repetition II	Repetition III	
Negative control (DMSO)	0	0	0	0
Dersono water apple Leaf Acetone Fraction 6.25%	13.27	0	0	4.42±6.25
Dersono water apple Leaf Acetone Fraction 12.5%	10.14	0	0	3.38±4.78
Dersono water apple Leaf Acetone Fraction 25%	14.01	9.54	10.2	11.25±1.97
Dersono water apple Leaf Acetone Fraction 50%	13.32	11.4	11.51	12.08±0.88
Positive control (amoxicillin)	45.16	39.19	39.46	41.27±2.75

Blood agar media is a good medium for the growth of S. Pyogenes bacteria. The formation of inhibition zones in the S. Pyogenes bacterial culture media indicates an antibacterial effect. A comparison of inhibition zones in the ethanol extract group (A) and the acetone fraction group (B) in this study is shown in Figure 1 below.

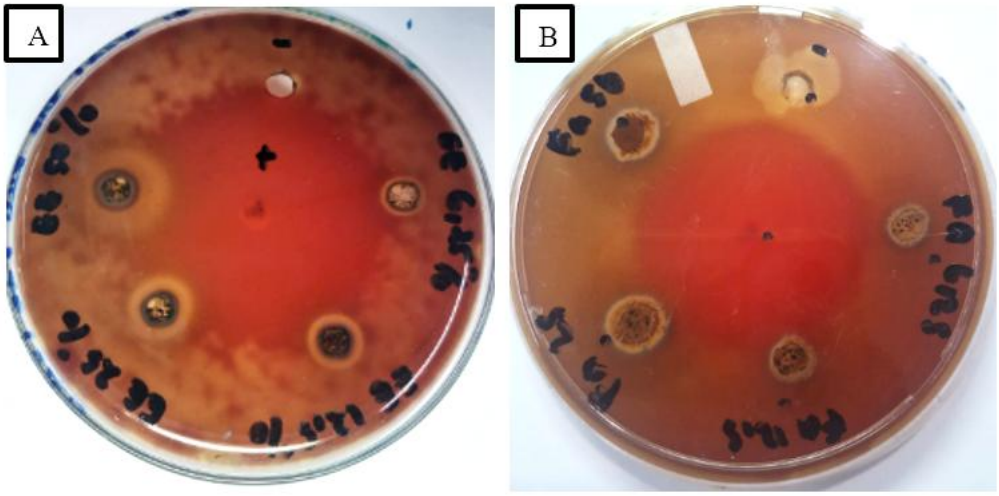

Figure 1: Growth inhibition zone of *Streptococcus pyogenes* bacteria on blood agar media. A. Ethanol Extract of Dersono water apple Leaves (*Syzygium malaccense*), B. Acetone Fraction of Dersono water apple Leaves (*Syzygium malaccense*)

In clinical identification of S. pyogenes, blood agar culture media is used. S. pyogenes colonies are dome-shaped with clear edges and a smooth or moist surface, after incubation for 24 hours at 35–37°C. With a diameter of more than 0.5 mm and a grayish-white appearance, these colonies are surrounded by a zone of β-hemolysis that is often two to four times the diameter of the colony [9,10].

Phytochemical test data of ethanol extract and acetone fraction of Dersono water

apple (*Syzygium malaccense*) leaves can be seen in Table 3. The phytochemical test examination method is based on the method used by Taufiq [11].

Table 3. Secondary Metabolite Content of Ethanol Extract and Acetone Fraction of Dersono Water Apple Leaves (*Syzygium malaccense*)

No	Active Ingredients Content	Ethanol Extract	Acetone Fraction
1	Flavonoids (positive if magenta red color forms)	-	-
2	Saponin (positive if foam forms)	+	+
3	Tannin (positive if a bluish black/green color forms)	+	+
4	Wagner alkaloids (positive if reddish brown color forms)	+	+

The data obtained from this study were tested for normality using the Shapiro-Wilk test and obtained a p value <0.05 meaning the data was not normal, while the homogeneity test with the Levene test obtained a p value <0.05 meaning the data was not homogeneous. The Non-parametric Kruskal Wallis and obtained the Ethanol Extract value p <0.05 and the Acetone Fraction p <0.05, at least there was 1 significantly different group. Furthermore, the post-hoc test with Mann-Whitney to see the significant differences between groups. Based on table 4 was found that Ethanol Extract 6,25%; 12,5%; 25%; and 50% were different significant with negative control. It was show that they have antibacterial effect on S. pyogenes. Based on table 5 was found that Aceton Fraction 6,25% and 12,5 % not significant different with negative control. Although there was a zone of inhibition (table 2), but the inhibition zone number was too small, so it was considered statistically insignificant. Acetone fraction 25% and 50% was significant different with negative control and it was show that Acetone fraction 25% and 50% have antibacterial effect. The level of antibacterial effectiveness can be determined by measuring the percentage (%) of inhibitory power using the formula[31] (table 6):

$$E = \frac{D}{Da} \; x \; 100$$

Information:

E: Antibacterial effectiveness (%)

D: Inhibition zone diameter (mm)

Da: Diameter of antibiotic inhibition zone (mm)

Table 4. *Mann-Whitney* Test of Ethanol Extract of Dersono Water Apple Leaves (*Syzygium malaccense*)

No	Comparison of Test Groups	*p* value	Difference
1	DMSO - Amoxicillin	0.035	significantly different
2	DMSO - Ethanol Extract 6,25%	0.037	significantly different
3	DMSO - Ethanol Extract 12,5%	0.037	significantly different
4	DMSO - Ethanol Extract 25%	0.037	significantly different
5	DMSO - Ethanol Extract 50%	0.037	significantly different
6	Ethanol Extract 6,25% - Ethanol Extract 12,5%	0.127	not significantly different
7	Ethanol Extract 6,25% - Ethanol Extract 25%	0.050	not significantly different
8	Ethanol Extract 6,25% - Ethanol Extract 50%	0.050	not significantly different
9	Ethanol Extract 12,5% - Ethanol Extract 25%	0.127	not significantly different
10	Ethanol Extract 12,5% - Ethanol Extract 50%	0.050	not significantly different
11	Ethanol Extract 25% - Ethanol Extract 50%	0.050	not significantly different
12	Amoxicillin - Ethanol Extract 6,25%	0.046	significantly different
13	Amoxicillin - Ethanol Extract 12,5%	0.046	significantly different
14	Amoxicillin - Ethanol Extract 25%	0.050	not significantly different
15	Amoxicillin - Ethanol Extract 50%	0.050	not significantly different

Table 5. *Mann-Whitney* Test of Acetone Fraction of Dersono Water Apple Leaves (*Syzygium malaccense*)

No	Comparison of Test Groups	*p* value	Difference
1	DMSO - Amoxicillin	0.037	significantly different
2	DMSO - Acetone Fraction 6,25%	0.317	not significantly different
3	DMSO - Acetone Fraction 12,5%	0.317	not significantly different
4	DMSO - Acetone Fraction 25%	0.037	significantly different
5	DMSO - Acetone Fraction 50%	0.037	significantly different
6	Acetone Fraction 6,25% - Acetone Fraction 12,5%	0.796	not significantly different
7	Acetone Fraction 6,25% - Acetone Fraction 25%	0.268	not significantly different
8	Acetone Fraction 6,25% - Acetone Fraction 50%	0.268	not significantly different
9	Acetone Fraction 12,5% - Acetone Fraction 25%	0.121	not significantly different
10	Acetone Fraction 12,5% - Acetone Fraction 50%	0.046	significantly different
11	Acetone Fraction 25% - Acetone Fraction 50%	0.513	not significantly different
12	Amoxicillin - Acetone Fraction 6,25%	0.046	significantly different
13	Amoxicillin - Acetone Fraction 12,5%	0.046	significantly different
14	Amoxicillin - Acetone Fraction 25%	0.050	not significantly different
15	Amoxicillin - Acetone Fraction 50%	0.050	not significantly different

Based on table 6, the antibacterial strength of Dersono water apple leaf ethanol extract is strong to very strong, while the acetone fraction is moderate to very strong. From these results, it was found that the inhibitory power of the ethanol extract was better than the acetone fraction. The use of acetone (semi-polar) and ethanol (polar) solvents is because these two types of solvents are volatile, safer for the environment compared to other solvents, can extract compounds in plants better, and are the solvents most often used to extract a material.

Table 6. Antibacterial Power of Ethanol Extract and Acetone Fraction of Dersono water apple Leaves

No	Test materials and concentrations	Inhibitory Power (%)	Antibacterial Power
1	Ethanol Extract 6,25%	19.56	strong
2	Ethanol Extract 12,50%	23.11	very strong
3	Ethanol Extract 25%	28.31	very strong
4	Ethanol Extract 50%	39.61	very strong
5	Acetone Fraction 6.25%	10.72	moderate
6	Acetone Fraction 12.50%	8.19	moderate
7	Acetone Fraction 25%	27.26	very strong
8	Acetone Fraction 50%	29.26	very strong

Based on table 1, table 2, table 4 and table 5 it can be seen that the ethanol extract and acetone fraction of Dersono water apple leaves have the effect of inhibiting the growth of *Streptococcus pyogenes* bacteria.

Al-Zabt et al., (2022) explains the antibacterial mechanism of *Syzygium malaccense* is an increase in intracellular material leakage due to the release of nucleic acids (DNA and RNA) and cellular protein leakage. The antibacterial power on bacterial membranes depends on the concentration[12]. Based on table 3, the secondary metabolite content of the ethanol extract and acetone fraction is obtained in the form of saponins, tannins, and alkaloids.

Saponins contain carbohydrate, and aglycones. Based on the chemical structure of their aglycones, saponins are classified as triterpenes (C30) and steroids (C27). Saponins are abundant in higher plants [13,14]. Alina et al. (2023) stated that saponins are obtained by extracting plant parts (roots, stems, bark, leaves, shells, or seeds) with solvents (ethanol, methanol, or water) containing saponin[13]. Pharmacological properties of saponins include antibacterial, molluscicidal, insecticidal, antibacterial, cytotoxic, and hemolytic properties[13]. The antibacterial activity of saponins is based on their interaction with cell membranes[14]. The mechanism of action of saponins is by binding to lipids, increasing the permeability of bacterial membranes and allowing them to enter bacterial cells[15].

Tannins are polyphenols that possess anti-inflammatory, antibacterial, antioxidant, anticancer, and nutrient absorption-inhibiting and cardioprotective properties[16]. Tannins (tannic acid) have antibacterial effects against Gram-positive and Gram-negative bacteria, including Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes, Enterococcus faecalis, Pseudomonas aeruginosa, Yersinia enterocolitica, and Listeria innocua[17]. Tannins have several antibacterial mechanisms of action, including iron chelation, inhibition of bacterial cell wall synthesis, causing cell membrane rupture, and inhibition of the fatty acid biosynthesis pathway, thus disrupting bacterial cell metabolism [17, 18]. Tannins are more effective against Gram-positive bacteria than Gram-negative bacteria. This happens because Gram-negative bacteria have a 2-layer cell membrane[17].

Alkaloids are secondary metabolites containing at least one nitrogen atom. Alkaloids have diverse pharmacological properties, including analgesic, antibacterial, antimalarial, anticancer, and antihyperglycemic activities[16, 19, 20]. Alkaloids are a potential class of natural antibiotics. They have a broad range of antibacterial activity, rarely cause side effects, and have a low tendency to develop drug resistance. The antibacterial mechanisms of alkaloids include preventing bacterial cell wall formation, altering cell membrane permeability, disrupting bacterial metabolism, and inhibiting protein and nucleic acid synthesis[21].

Syzygium malaccense (L.) Merr. & L.M.Perry (Myrtaceae) is an herb that has the potential to treat infections caused by *Streptococcus pyogenes* in the respiratory organs, reproductive organs, uropoetic organs, and skin infections[22]. *Syzygium malaccense* (L.) Merr. Fruit has antioxidant activity, bark has antiviral activity, and there are antifungal, antibacterial, anti-inflammatory, antioxidant, and cytotoxic (in vitro) properties[16].

The combination of the effectiveness of secondary metabolites such as alkaloids, tannins, and saponins, contributes to eradicating *Streptococcus pyogenes* bacteria effectively. This reseach was different with Mellin (2017) explained that Dersono water apple (*Syzygium malaccense* (L.) Merr & L.M. Perry) was a plant belonging to the Myrtaceae family that contains a lot of flavonoids. The results of the study showed that the flavonoid content of ethanol extract of guava leaves and the n-hexane fraction, chloroform fraction and ethanol fraction were 1.73 ± 0.44%, 2.47 ± 0.38%, 2.61 ± 0.16% and 1.37 ± 0.26% respectively. The results showed that the chloroform fraction had a higher flavonoid content than the others[23].

Differences in the content of secondary metabolites including flavonoids in a plant are influenced by where the plant grows, the drying process, the extraction process, the use of solvents, and temperature[24, 25, 26,27]. The presence and concentration of various secondary metabolites influence the minimum inhibitory concentration (MCI) against bacteria[13]. The content of secondary metabolites in the ethanol extract and acetone fraction were contributed together as antibacterials against S. pyogenes. With increasing concentration in the sample, the

diameter of the inhibition zone and inhibitory power were increases, all sample concentrations have the potential as antibacterial.

CONCLUSION

All treatment groups ethanol extract and acetone fraction of *Syzygium malaccense* have antibacterial effects against Streptococcus pyogenes. Ethanol extract and acetone fraction of Dersono water apple leaves contain saponins, tannins and alkaloids. The Suggestions for further research to conduct quantitative phytochemical tests to determine the exact levels of each secondary metabolite.

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