

Activity of Water and Ethanol *Syzygium myrtifolium* Walp. as an Antibacterial *Aggregatibacter actinomycetemcomitans*

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ABSTRACT

Background: One of the pathological conditions in the mouth that affects periodontal tissue is periodontitis. *Aggregatibacter actinomycetemcomitans* is a potential trigger of periodontal disease if allowed to settle, multiply, and express virulence factors. The pharmacological activity of *Syzygium myrtifolium* based on solvent extract has several functions, one of which is antibacterial. Solvents function to attract compounds or active ingredients in natural materials, and solvents are distinguished based on their polarity. Water is very polar, so it will attract compounds or active ingredients that are very polar, while ethanol is polar, so it will attract active substances/compounds that are polar. So that later it can be known how both perform. **Objective:** This study aims to determine the activity of water and ethanol extracts of *Syzygium myrtifolium* Walp. 10%, 20%, 40%, and 80% in inhibiting the growth of *Aggregatibacter actinomycetemcomitans* bacteria. **Materials and Methods:** This study is a laboratory experiment. Ethanol extract of *Syzygium myrtifolium* Walp. was partitioned to obtain a water extract of *Syzygium myrtifolium* Walp. A disc diffusion method on Mueller Hinton Agar (MHA) media was the antibacterial test method used in this study (n=4). Metronidazole 50 µg was used as a positive control and DMSO 2% as a negative control. **Results:** The average inhibition zone of ethanol extract was 9.11 mm, 11.475 mm, 13.625 mm, and 15.2875 mm, respectively, while the water extract was 10.3125 mm, 13.25 mm, 14.725 mm, and 16.725 mm, respectively. Positive control 22.975 mm and negative control 0 mm. **Conclusion:** Water and ethanol extracts of *Syzygium myrtifolium* Walp. have antibacterial activity against *Aggregatibacter actinomycetemcomitans*.

Keywords: *Aggregatibacter Actinomycetemcomitans*, *Syzygium myrtifolium* Walp., Antibacterial Activity

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INTRODUCTION

Aggregatibacter actinomycetemcomitans on the mucosa is considered a risk factor for the translocation of the organism to the gingival margin. This bacteria is a potential trigger of periodontal disease if allowed to persist, proliferate, and express virulence factors. These bacteria are early colonizers in the disease process and reject oxygen and hydrogen peroxide.¹

One of the pathological conditions that occurs in the mouth and affects the periodontal tissue or supporting teeth is periodontitis.² Periodontitis is a multifactorial disease triggered by an imbalance between environmental factors and the host defense. The environmental factors include infectious disease components, such as pathogenic bacteria in dental plaque, and lifestyle. The innate immune system in the host response may become highly reactive and result in an excessive inflammatory response.³

The prevalence of periodontal disease in the world is around 20 – 50%, and it is a continuous increase in prevalence every year.⁴ Periodontal disease in Indonesia ranks second after tooth decay, reaching a percentage of 96.58%.⁵ Based on 2018 Basic Health Research Data in Indonesia, the overall prevalence of periodontitis reached 74.1% based on certain characteristics, while only 0.4% of the population received gum or periodontal treatment.⁶ In the Central Java region, periodontal disease has a prevalence of 25.8%⁴.

Treatment and prevention of periodontal disease can be surgical and non-surgical. Various periodontal surgical procedures are used to treat chronic periodontitis, one of which is regenerative periodontal treatment using bone graft and platelet-rich fibrin (PRF).⁷ Non-surgical periodontal treatment procedures such as scaling root planing and administering antibiotics. Scaling root planing is an invasive procedure on the surface of the tooth root.⁸ The nature of the antibiotic is given for specific

pathogens in periodontal tissue, allogenic, non-toxic, and substantive.⁹

The use of synthetic drugs can cause negative impacts, both immediately and in the longer term. Adequate antibacterial administration is necessary to treat bacterial infections. However, increasing resistance to the widespread use of antibacterials has become a major public health problem worldwide. Therefore, it is necessary to search for new antibacterial agents to treat infections without causing resistance.¹⁰ Meanwhile, herbal medicines may cure diseases with minimal side effects because they are made from natural ingredients.¹¹ *Syzygium myrtifolium* Walp. has potential as a source of medicine. *Syzygium myrtifolium* Walp. belong to the Myrtaceae family.¹²

Pharmacological activity of *Syzygium myrtifolium* Walp. based on the solvent extract, it involves several functions, one of which is as an antibacterial agent.¹³ The abilities of *Syzygium myrtifolium* Walp. as an inhibitor of bacterial growth due to the content of tannins, saponins, flavonoids, and essential oils.¹⁴ In addition, the phenolic and flavonoid content is high in the ethanol and ethyl acetate extracts from *Syzygium myrtifolium* Walp. indicates that both have antioxidant properties and can inhibit the angiogenesis process.^{12,14}

Research conducted by Syafrana & Wiranti, 2022¹⁵ shows that the green and red leaves of *Syzygium myrtifolium* Walp. extract using ethanol has potential antibacterial properties against *S. mutans*. Both extracts can inhibit the growth of *S. mutans* bacteria at concentrations of 10%, 20%, 40%, and 80%. The inhibition values of the two extracts against *S. mutans* at each concentration were not significantly different, and the minimal inhibitory concentration could not be obtained.

Based on the description above, researchers are interested in investigating the antibacterial effects of extracts from *Syzygium myrtifolium* Walp. against the growth of *Aggregatibacter actinomycetemcomitans*

bacteria. This is because these bacteria are included in the type of pathogenic bacteria that is the main cause of periodontitis in the mouth. Researchers used extract solvents from water and ethanol. Solvents function to attract compounds or active ingredients present in natural materials, and solvents are differentiated based on polarity. Water is very polar and will attract compounds or active ingredients that are very polar. While ethanol is polar, it will attract active substances. So you can see later how the two of them are doing. Thus, it is expected that the water and ethanol extract of *Syzygium myrtifolium* Walp. can be an alternative for preventing and treating health problems in the oral cavity.

MATERIALS AND METHODS

This type of research was an experimental laboratory with a post-only control group design. The sample was *Aggregatibacter actinomycetemcomitans* (ATCC 43718).

Four repetitions by randomly selecting *Aggregatibacter actinomycetemcomitans* bacteria in Mueller Hinton Agar (MHA) media petri dishes then dividing them into *Aggregatibacter actinomycetemcomitans* groups with water and ethanol extract treatment of *Syzygium myrtifolium* concentrations of 10%, 20%, 40% and 80% negative control using DMSO 2% and positive control with Metronidazole 50 µg.

The inhibition zone test results that were obtained were then carried out statistical testing using *Kruskal-Wallis* test followed by *Mann Whitney*.

RESULTS

Tables 1 and 2 show the chemical content of water extract *Syzygium myrtifolium* and the chemical content of ethanol extract *Syzygium myrtifolium*

Table 1. Chemical content of water extract *Syzygium myrtifolium* Walp.

Compound Name	Alkaloid	Flavonoid	Tanin	Fenolik	Steroid	Terpenoid	Saponin
test method	ammoniacal chloroform + H ₂ SO ₄ 2N + Dragendorff	Mg powder + alcohol: HCL + amyl alcohol	+FeCl ₃ 1% 2 drops	+FeCl ₃ 5% 2 drops	H ₂ SO ₄ 2 drops + Anhydro us Acetic Acid 1 drop (Lieberman burchad (LB) test)	H ₂ SO ₄ 2 drops + Anhydro us Acetic Acid 1 drop (Lieberman burchad (LB) test)	+H ₂ O
Before Adding Reagents							
After Added Reactor							
Test results	+	-	+	+	+	-	+

Based on phytochemical testing on the water extract of *Syzygium myrtifolium* Walp., the water extract of *Syzygium myrtifolium* Walp. contains alkaloids, tannins, phenolics, steroids, and saponins (Tabel 1).

Table 2. Chemical Contents of *Syzygium myrtifolium* Walp ethanol extract.

Compound Name	Alkaloid	Flavonoid	Tanin	Fenolik	Steroid	Terpenoid	Saponin
test method	ammoniacal chloroform + H ₂ SO ₄ 2N + Dragendorff	Mg powder + alcohol: HCL + amyl alcohol	+ FeCl ₃ 1% 2 drops	+FeCl ₃ 5% 2 drops	H ₂ SO ₄ 2 drops + Anhydrous Acetic Acid 1 drop (Lieberman burchad (LB) test)	H ₂ SO ₄ 2 drops + Anhydrous Acetic Acid 1 drop (Lieberman burchad (LB) test)	+H ₂ O
Before Adding Reagents							
After Added Reactor							
Test result	+	+	+	+	+	+	+

Based on phytochemical testing on the ethanol extract of *Syzygium myrtifolium* Walp., the ethanol extract of *Syzygium myrtifolium* Walp. contains alkaloids, flavonoids, tannins, phenolics, steroids, terpenoids, and saponins (Tabel 2).

Figure 1 shows the inhibition zone results formation. Tables 3 and 4 show the mean inhibition chemical content of water extract *Syzygium myrtifolium* and the chemical content of ethanol extract *Syzygium myrtifolium*.

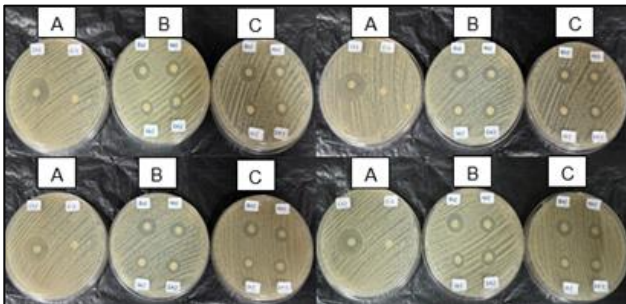


Figure 1. Bacterial Inhibition Zone Results Information:

A: Positive Control and Negative Control

B: *Syzygium myrtifolium* Walp Water Extract.

C: *Syzygium myrtifolium* Walp Ethanol Extract.

Table 3. Mean inhibition zone results of *Syzygium myrtifolium* Walp water extract.(mm)

No	K (+)	K (-)	10%	20%	40%	80%
1	22,95	-	9,15	11,60	13,80	15,20
2	23,20	-	9,05	11,35	13,75	15,40
3	22,80	-	9,20	11,40	13,40	15,35
4	22,95	-	9,05	11,55	13,55	15,20
Mean	22,975	-	9,11	11,475	13,625	15,2875
Std. Dev	0,16583	-	0,07500	0,11902	0,18484	0,10308

Table 4. Mean inhibition zone results of *Syzygium myrtifolium* Walp ethanol extract.

No	K (+)	K (-)	10%	20%	40%	80%
1	22,95	-	10,40	13,40	14,95	16,75
2	23,20	-	10,20	13,05	14,80	16,60
3	22,80	-	10,05	13,20	14,60	16,40
4	22,95	-	10,60	13,35	14,55	16,35
Mean	22,975	-	10,3125	13,25	14,725	16,525
Std. Dev	0,16583	-	0,23936	0,15811	0,18484	0,18484

Tabel 5. The statistical analysis results of *Syzygium myrtifolium* Walp water extract.

	water 80%	water 40%	water 20%	water 10%	positive control	negative control
water 80%		0,020	0,020	0,019	0,019	0,013
water 40%			0,021	0,020	0,020	0,014
water 20%				0,020	0,020	0,014
water 10%					0,019	0,013
positive control						0,013
negative control						

Tabel 6. The statistical analysis results of *Syzygium myrtifolium* Walp ethanol extract.

	etanol 80%	etanol 40%	etanol 20%	etanol 10%	positive control	negative control
etanol 80%		0,021	0,021	0,021	0,020	0,014
etanol 40%			0,021	0,021	0,020	0,014
etanol 20%				0,021	0,020	0,014
etanol 10%					0,020	0,014
positive control						0,013
negative control						

Tables 5 and 6 above show the results of the Mann-Whitney test to see the differences in inhibition zone test results between treatment groups in all group comparisons. Data can be said to have a significant difference if it has a p-value < 0.05. Based on tables 11 and 12, it can be seen that the results of the Mann-Whitney test for all group comparisons show a p-value < 0.05, so it can be interpreted that there is a significant difference between groups in the inhibition zone data for water and ethanol extracts of *Syzygium myrtifolium* Walp. on the growth of *Aggregatibacter actinomycetemcomitans* bacteria.

DISCUSSIONS

Based on the data presented, there are differences in the effectiveness of the concentrations of water extract and ethanol extract from *Syzygium myrtifolium* Walp.) with varying concentrations of 10%, 20%, 40%, and 80%. Tables 3 and 4 showed highest average diameter of the inhibition zone was recorded in the control group using Metronidazole, with a size reaching 22.9 mm. However, in the negative control group using 2% DMSO, there was no visible zone of inhibition because the result was 0.

The average growth inhibition value of *Aggregatibacter actinomycetemcomitans* bacteria by water extract of *Syzygium myrtifolium* Walp. showed variations between treatment groups. The treatment group with 10% water extract had the lowest average resistance value, namely 9.11 mm, while the treatment group with 80% water extract showed the highest average resistance value, reaching 15.28 mm. Meanwhile, in table 4, the treatment group with ethanol extract shows a similar pattern, where the 10% concentration has the lowest average resistance value, namely 10.31 mm, and the 80% ethanol concentration shows the highest average resistance value, namely 16.52 mm. From the results of this research, it can be concluded that the higher the concentration of water extract and ethanol extract from *Syzygium myrtifolium* Walp., the higher their ability as an antibacterial agent against *Aggregatibacter actinomycetemcomitans*.

The width of the inhibition zone is classified into four categories, namely: activity is weak (<5mm), moderate (6-10mm), strong (11-20mm) and very strong (>21mm). This means that bacterial growth is unable to withstand the antibacterial process produced by the extract (*Syzygium myrtifolium* Walp.) so that an inhibition zone is formed. From table 3 it can be seen that the average inhibitory power of water extract of *Syzygium myrtifolium* Walp. with a

concentration of 10% is in the medium category, while at concentrations of 20%, 40% and 80% it is in the strong category. In table 4 it can be seen that the average ethanol extract of *Syzygium myrtifolium* Walp. with a concentration of 10% is in the medium category, while at concentrations of 20%, 40% and 80% it is in the strong category.

This study used a positive control, namely 50 µg metronidazole. Metronidazole is known to have very strong antibactericidal properties. The mechanism of action of metronidazole in killing bacteria involves the penetration of the compound into microorganisms. After entering the microorganism, metronidazole is reduced and produces a polar product, namely 2-hydroxymethyl metronidazole. This compound then interacts with bacterial DNA, causing disruption of the DNA helix structure, and inhibiting nucleic acid synthesis, ultimately resulting in bacterial cell death.¹⁶

The effectiveness of an antibacterial substance in inhibiting and killing bacteria can be influenced by several factors, including the type of compound being tested, its concentration and the type of bacteria that is targeted. Phytochemical analysis in the laboratory shows that water and ethanol extracts from *Syzygium myrtifolium* Walp. contain various bioactive compounds. Water extract from *Syzygium myrtifolium* Walp. is known to contain alkaloids, tannins, saponins, phenolics, steroids and terpenoids, while ethanol extract from *Syzygium myrtifolium* Walp. contains alkaloids, flavonoids, tannins, saponins, phenolics and steroids. Based on these findings, both types of extracts show potential as antibacterial agents because they contain these bioactive compounds.

Antibacterial activity begins with terpenoid compounds, which damage and reduce the permeability of bacterial cell walls, resulting in nutritional deficiencies in the bacteria. The next involves flavonoids, which form extracellular complexes with proteins, followed by steroid compounds that damage lipid membranes, causing liposome leakage. The

saponin compound then causes leakage of proteins and enzymes in the bacterial cells. Alkaloid compounds inhibit the peptidoglycan component in bacterial cells, disrupting the formation of perfect cell walls. After that, tannins disrupt the stability of the bacterial cell membrane by removing important components such as proteins and nucleic acids from the bacterial cell. The last compound, phenol, plays a role in damaging cell walls by destroying the enzymes in bacteria. Thus, this process causes the bacteria to lack nutrition and even kills the bacteria until they die.

In this study, this significant difference was caused by variations in the concentration of water and ethanol extract from *Syzygium myrtifolium* Walp. Differences in the concentration of antimicrobial substances influence the diameter of the inhibition zone, where the higher the concentration of the substance in the extract, the larger the diameter of the bacterial inhibition zone produced.

CONCLUSION

Based on the results of research on the activity of *Syzygium myrtifolium* Walp. water and ethanol extract as antibacterial, *Aggregatibacter actinomycetemcomitans*, it can be concluded that extract of *Syzygium myrtifolium* Walp. water and ethanol fractions have antibacterial activity with concentration. 10%, 20%, 40%, 80% against *Aggregatibacter actinomycetemcomitans* bacteria.

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