



ANTIMICROBIAL TEST OF DAYAK ONIONS (ELEUTHERINE PALMIFOLIA (L.) MERR) LEAVES ON THE GROWTH OF STREPTOCOCCUS MUTANS

Sumarni¹, Indah Kumalasari^{2*}

^{1,2} Medicine and Health Science Faculty, Muhammadiyah University of Makassar
¹sumacardio@med.unismuh.ac.id, ²indahkumalasari@med.unismuh.ac.id

Abstract

Streptococcus mutans bacteria is a gram-positive bacterium that can cause infective endocarditis. In USA, the incidence of endocarditis ranges from 3 to 10 cases per 100,000 population per year. Infections caused by bacteria can be treated with antibiotics. Dayak onion leaves have been used as medicinal ingredients because they contain phytochemicals such as flavonoids, saponins, alkaloids, tannins and triterpenoids which act as antibacterial. This study aims to identify the antibacterial properties of Dayak onion leaves extract against *Streptococcus mutans* bacteria in vitro. This study used a true experimental design with the treatment of Dayak onion leaves against *Streptococcus mutans* bacteria to test its antibacterial activity using the well diffusion method with concentrations of 25%, 50%, and 75%. The average measurement results with concentrations of 75%, 50%, and 25% were 25,41 mm, 23,20 mm, and 22,32 mm respectively. The positive control used in the experiment was the Amoxicillin antibiotic with an average inhibition of 18,02 mm and the negative control was Ampicillin antibiotic with an average inhibition of 10,14 mm. Meanwhile, the placebo control was 10% DMSO without an inhibition zone for bacteria. Based on the study results, the extract of Dayak onion leaves with a concentration of 25%, 50%, and 75% have antibacterial activity against *Streptococcus mutans* bacteria.

Keywords: *Extract of Dayak onions; Well diffusion method; Streptococcus mutans*

@Jurnal Ners Prodi Sarjana Keperawatan & Profesi Ners FIK UP 2025

□ Corresponding author :

Address : Makassar, Sulawesi Selatan, Indonesia

Email : indahkumalasari@med.unismuh.ac.id

INTRODUCTION

Streptococcus mutans is gram-positive bacteria in the *Streptococcus viridans* group. Generally, this type of bacteria is harmless and can be found in the human oral cavity^{1,2}. However, it can cause infection if it enters the bloodstream from invasive actions of the teeth, throat, digestive tract and other invasive actions, as well as from the use of non-sterile injection such as drug users^{3,4}.

Streptococcus mutans is the causative agent of infective endocarditis (IE) which can be transmitted through saliva or direct contact⁵. Infective endocarditis (IE) is considered a systemic septic disease in which vegetation containing platelets, fibrin, inflammatory cells, and bacteria are formed on the heart valves.

The incidence of infective endocarditis in the USA is estimated to reach 3-10/100,000 people/year. This figure increased over the past two decades. Hospital mortality rates are high, ranging from 14 to 22%, with high annual mortality rates of 15-30%. *Staphylococcus aureus* becomes the most common cause of IE with a prevalence of 26.6% of all cases, followed by *Streptococcus viridans* group at 18.7%⁶. *Streptococcus mutans* has shown resistance to some antibiotics such as erythromycin, linomycin, penicillin, and ampicillin. Therefore, it is important to explore new antimicrobial compounds with higher potential in inhibiting the growth such as extracts of Dayak onion leaves, a wild plant that is widely utilized⁷.

Dayak onions (*Eleutherine palmifolia* (L.) Merr.) are a typical herbal plant in Central Kalimantan that has been used by the Dayak people as a traditional medicine for generations. Generally, they only use the bulb and throw out the leaf⁸. Previous studies have identified the efficacy of Dayak onion leaves on the growth of *Candida albicans* and found that the leaves contain antibacterial compounds such as alkaloids, flavonoids, saponins, phenols, tannins, and triterpenoids^{9,10}. The chemical compounds

have the potential to be antioxidants, especially flavonoids, which can prevent the growth of free radicals in the body while repairing damaged body cells. Flavonoids also function as anti-inflammatories and antibiotics¹¹. Therefore, the metabolite compounds contained can inhibit the growth of *Streptococcus mutans* bacteria.

METHODS

Tools and Materials

This study used some tools such as an Erlenmeyer flask, measuring cup, chemical glass, test tube, test tube rack, dropper, water bath, analytical balance, extraction flask, stirring rod, stirrer, petri dish, rotary evaporator (oven), loop needle, tweezers, incubator, laminar air flow, vial, autoclave, micropipette, cup cylinder, graduated ruler, caliper, and photography equipment.

The materials used in this study were Dayak onion leaf extracts, *Streptococcus mutans* bacteria, 10% Dimethyl sulfoxide solution (10% DMSO), 96% ethanol, blank disc antibiotic Ampicillin 10, blank disc antibiotic Amoxicillin 30, Nutrient Agar (NA), filter paper No. 1, label paper and aluminum foil.

Extraction of Dayak Onion Leaves

Dayak onion leaves (*Eleutherine palmifolia* (L.) Merr) obtained from Tumbit Dayak, East Kalimantan, were wet sorted to separate foreign objects. Then, they were washed with running water. They were cut into small pieces dried by airing and protected from sunlight. They were blended to get smooth textures and then the dried simplicia was weighed.

The extraction was carried out using the maceration method by storing 200 g of simplicia in a jar, then adding 96% ethanol solvent \pm 2 L of simplicia that had been stored in the container. Then, it was closed tightly and stirred every 24 hours for 3 days to get the extract from the simplicia that had been soaked for 3 days. The filtration process was to separate the dregs so that a wet extract was

obtained. After getting the wet extract, the evaporation process was continued with a rotary evaporator and obtained 41 grams of thick extract of Dayak onion leaves.

Preparation of Concentration

Dilution was carried out to produce some concentrations of Dayak onion leaf extracts (*Eleutherine palmifolia* (L.) Merr) and to see its effect in inhibiting the growth of *Streptococcus mutans* bacteria. The dilutions were 25%, 50%, and 75% using 10% DMSO solvent.

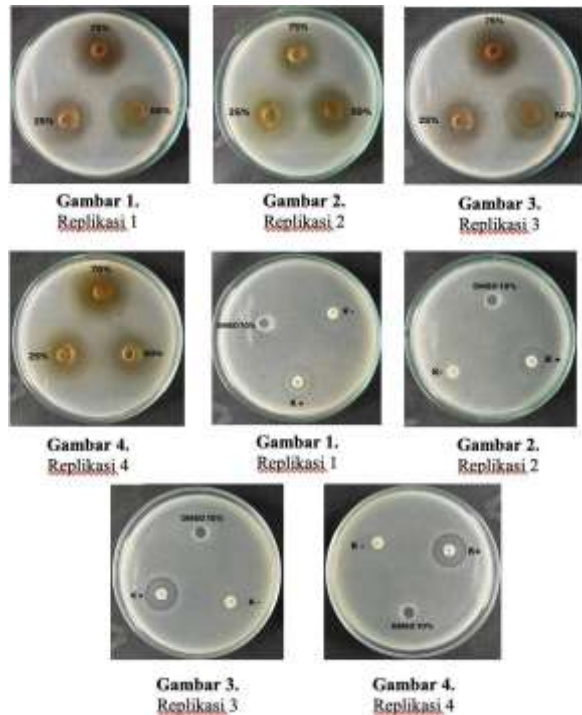
Antibacterial Activity Testing

The rejuvenated *Streptococcus mutans* bacteria were then inoculated into a petri dish containing Nutrient agar (NA) media. Then, the researcher made a hole using a cylindrical cup and inserted the Dayak onion leaf extract (*Eleutherine palmifolia* (L.) Merr) with concentrations of 25%, 50%, and 75% into the petri dish. Amoxicillin was the positive control and Ampicillin was the negative control with 10% DMSO as a placebo control. The next, it was incubated for 24 hours at a temperature of 37°C. The incubation results in the form of a clear zone in the hole indicating the presence or absence of bacterial growth.

Inhibition Zone Measurement

The researcher used a caliper to measure the size of the inhibition zone formed around the hole. The distance was measured based on the diameter of the inhibition zone formed and expressed in millimeters.

RESULTS AND DISCUSSION



The antibacterial activity test of Dayak onion leaf extract (*Eleutherine palmifolia* (L.) Merr) against *Streptococcus mutans* bacteria used the well method with concentrations of 25%, 50% and 75%, which was grown in Natrium Agar media to form the inhibition zone in the growth of the bacteria. Besides, observations were also made using positive controls (Amoxicillin), negative controls (Ampicillin) and placebo controls (DMSO 10%). Repetition was carried out 5 times based on the Frederrer formula. The inhibition zone was measured based on the diameter using a caliper. The results of the inhibition measurement are as follows.

Table 1. Results of Inhibition Zone Measurement Formed in NA Media

Konsentrasi(%)	Diameter Zona Hambat				Rata-rata
	1	2	3	4	
25%	22,54	22,41	22,15	22,18	22,32
50%	23,38	23,16	23,14	23,14	23,20
75%	25,99	25,17	25,20	25,29	25,41
Kontrol Positif (Amoxicillin)	18,03	18,00	18,01	18,05	18,02
Kontrol Negatif (Ampicillin)	10,13	10,18	10,12	10,15	10,14
Kontrol Plasebo (DMSO 10%)	0	0	0	0	0

Based on Table 1, the test of Dayak onion leaf extract (*Eleutherine palmifolia* (L.) Merr) on the growth of *Streptococcus mutans* bacteria at a concentration of 25% obtained an average inhibition zone of 22.32 mm. Meanwhile, at concentrations of 50% and 75%, it was 23.20 mm and 25.41 mm respectively.

The table above also shows that the control test as a comparison to see the strength of the inhibition zone with a positive control using the antibiotic Amoxicillin 25 obtained an average inhibition of 18.02 mm. In comparison, the negative control used the antibiotic Ampicillin 10 and obtained an average inhibition zone of 10.14 mm. The placebo control DMSO 10% did not affect the growth of the bacteria.

Discussion

The researcher performed antibacterial activity tests of ethanol extract of Dayak onion leaves (*Eleutherine palmifolia* (L.) Merr) against *Streptococcus mutans* bacteria. The results showed antibacterial properties that can inhibit bacterial growth with higher inhibition power compared to the positive control, namely the antibiotic amoxicillin, which has a lower average diameter of the inhibition zone.

The antibacterial properties of Dayak onion leaf extract (*Eleutherine palmifolia* (L.) Merr) have secondary metabolite compounds and

chemical compounds. The metabolite compounds are alkaloids, flavonoids, saponins, phenols, tannins, and triterpenoids that can inhibit bacterial growth. The chemical compounds have the potential as antioxidants, especially flavonoids, which can prevent the growth of free radicals in the body while repairing damaged body cells¹¹.

The mechanism of antibacterial compounds is carried out by changing membrane permeability, inhibiting enzyme activity, damaging cell walls and disrupting protein synthesis from bacteria after the incubation process. Dayak onion leaves contain compounds that can inhibit bacterial growth. The phytochemical tests for Dayak onion leaf extracts show that there are safe natural compounds that can be used as antibacterial agents in inhibiting the growth of *Streptococcus mutans* bacteria¹². The alkaloid and flavonoid content can interfere with the peptidoglycan components in bacterial cells, so the cell wall layer is not formed completely resulting in cell death. Tannins also have antibacterial activity. The mechanism of action of tannin compounds in inhibiting bacterial cells is by denaturing bacterial cell proteins. The mechanism of action of saponins is by damaging cell membranes causing leakage of proteins and enzymes in the cells. The mechanism of phenol as an antibacterial is by denaturing cell proteins. Other content, namely triterpenoids as antibacterials, reacts with transmembrane proteins on the outer membrane of bacterial cell walls, forming strong polymer bonds, resulting in damage to transmembrane proteins^{9,10}.

Greenwood explained that the inhibition of bacterial growth is classified into 4, namely <10 mm for no inhibition, 10 - 15 mm for poor inhibition, 16 - 20 mm for moderate inhibition and > 20 mm for strong inhibition¹³. In this study, the 75% concentration showed the greatest inhibition with a strong inhibition category. At concentrations of 50% and 25%, the inhibition was smaller and classified as the strong category. Agustina et al. (2020)

observed the ethanol extract of Dayak onion leaves against *Escherichia coli* which is gram-negative bacteria and revealed that at a concentration of 100%, the average inhibition zone was 21.88 mm which is classified as a strong category¹⁴. Previous studies have shown that Dayak onion leaves can also inhibit the growth of gram-negative bacteria. Eka Kumalasari et al. (2021) conducted the antibacterial activity test of ethanol extract of Dayak Onion (*Eleutherine Palmifolia* (L.) Merr) against the growth of *Candida albicans*. They showed that at a concentration of 100%, the average inhibition zone was 21.06 mm which is classified as a strong category referring to the Greenwood classification¹⁰.

The control test used Amoxicillin as a positive control, Ampicillin 10 as a negative control, and DMSO 10% as a placebo control. According to the Clinical and Laboratory Standards Institute (CLSI), the positive control is classified as a sensitive antibiotic, namely >18 mm¹⁰. Naelaz Zukhruf Wakhidatul et al. (2020) studied the use of amoxicillin antibiotics against *Streptococcus mutans* and obtained an inhibition zone of 20.72 mm which is classified as sensitive based on the CLSI table¹⁶. The negative control used ampicillin, a beta-lactam antibiotic which provides inhibition with a resistant category of <11 mm referring to CLSI¹⁵. This occurs due to mutations in the *Penicillin-Binding-Protein* (PBP) enzyme, an enzyme that forms bacterial cell walls so the formation of bacterial cell walls will be inhibited, resulting in autolysis (self-destruction) and cell death¹⁷.

The diameter measurement of the inhibition zone from the agar diffusion test showed that the ethanol extract of Dayak onion leaves (*Eleutherine palmifolia* (L.) Merr) has different diameters of the inhibition zone due to the different concentrations of the extract. This indicates that the higher the concentration of Dayak onion leaves used, the greater the diameter of the inhibition zone formed. The increase in the concentration of the substance can increase the content of active compounds

which function as antibacterials.

CONCLUSION

Based on the result of this study, it can be concluded that:

1. Measurement of the inhibition zone of Dayak onion leaf extract (*Eleutherine palmifolia* (L.) Merr) with concentrations of 25%, 50%, and 75% show strong inhibition.
2. Extracts of Dayak onion leaves show activity as an antibacterial against *Streptococcus mutans* bacteria.

REFERENCES

- Lemos JA, Palmer SR, Zeng L, Wen ZT, Kajfasz JK, Freires IA, et al. HHS Public Access. 2019;7(1):1–26.
- Kondo Y, Hoshino T, Ogawa M, Hidaka K, Hasuwa T, Moriuchi H, et al. *Streptococcus mutans* isolated from a 4- year-old girl diagnosed with infective endocarditis. 2019;(June):534–40.
- Nomura R, Matayoshi S, Otsugu M, Kitamura T, Teramoto N. crossm Contribution of Severe Dental Caries Induced by *Streptococcus mutans* to the Pathogenicity of Infective Endocarditis.
- Lilly L.S. Valvular Heart Disease. In: *Patophysiology of Heart Disease*. Sixth Edition. Lippincott Williams & Wilkins. Philadelphia. 2015.
- Esra K, Nurhan O, Yilmaz AD. Vertical and Horizontal Transmission of *Streptococcus Mutans* and Effective Factors: An In Vivo Study. 2020;
- Chen H, Zhan Y, Zhang K, Gao Y, Chen L, Zhan J. The Global, Regional, and National Burden and Trends of Infective Endocarditis From 1990 to 2019: Results From the Global Burden of Disease Study 2019. 2022;9(March).
- Al-shami IZ, Al-hamzi MA, Al- shamahy HA, Lutf A, Abdul A. Efficacy of some

- Antibiotics against Streptococcus Mutans Associated with Tooth decay in Children and their Mothers. 2019;0-3.
- Warsiti W, Wardani SD, Ramadhan AA, Yuliani R. Uji Aktivitas Antibakteri Ekstrak Etanol Bawang Dayak (Eleutherine palmifolia (L.) Merr) Terhadap Bakteri Staphylococcus aureus. *Pharmacon J Farm Indones.* 2019;15(2):75-82.
- Kumalasari E, Agustina D, Ariani N. Uji Aktivitas Antibakteri Ekstrak Daun Bawang Dayak (Eleutherine Palmifolia Merr.) Terhadap Escherichia Coli. *J Insa Farm Indones.* 2020;3(1):75-84.
- Kumalasari E. Uji Aktivitas Ekstrak Etanol 70% Daun Bawang Dayak (Eleutherinepalmifolia, (L.) Merr) Terhadap Pertumbuhan Candida Albicans. *J Insa Farm Indones.* 2021;4(2):176-85.
- Seja Y, Ardana M, Aryati F. Pengaruh Suhu dan Lama Penyimpanan Ekstrak Bawang Dayak (Eleutherine americana L (Merr)) terhadap Aktivitas Antibakteri. *Proceeding Mulawarman Pharm Conf.* 2018;8(November):150-5
- Shirly Kumala, Gabriel. Aktivitas Antioksidan Metabolit Sekunder Kapang Endofit Dan Tanaman Bawang Dayak (Eleutherine Americana (Aubl.) Merr.) secara In Vitro. *Jurnal Farmasi Indonesia.* 2019;11(2).
- Greenwood, 1995, Antibiotics Susceptibility (Sensitivity) Test, Antimicrobial ant Chemoteraphy. Addison Wesley Longman INE, San Frasisco, USA.
- Agustina D, Ariani N. Uji Aktivitas Antibakteri Ekstrak Daun Bawang Dayak (eleutherine palmifolia Merr.) terhadap escherichia coli. *Jurnal Insan Farmasi Indonesia.* 2020;3(1):75-8.
- Weinstein MP. Performance standards for antimicrobial susceptibility testing. Clinical and Laboratory Standards Institute; 2021.
- Wakhidatul Kiromah NZ, Rahmatulloh W. Uji Aktivitas antibakteri Ekstrak Metanol Dan Akuades Daun Ganitri (Elaeocarpus Ganitrus Roxb.) Terhadap Bakteri streptococcus mutans. *Acta Pharmaciae Indonesia: Acta Pharm Indo.* 2020;8(2):89.
- Pacifici GM. Clinical Pharmacology of ampicillin. *Journal of Case Reports and Medical History.* 2022;2(1):1-8.