

THE POTENTIAL OF PURPLE LEAVES ETHANOL EXTRACT (*Graptophyllum pictum* L.) AGAINST THE GROWTH OF *Staphylococcus aureus* and *Candida albicans*

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ABSTRACT

The Potential of Purple Leaves Ethanol Extract (*Graptophyllum pictum* L.) against *Staphylococcus aureus* and *Candida albicans*. This study is an experimental study with a comparative approach, aimed at analyzing the potential of purple leaves (*Graptophyllum pictum* L.) by using in vitro method against *Staphylococcus aureus* and *Candida albicans*. The clear zone was formed between the outer sides of the paper disc containing the ethanolic extract of the purple leaves (*Graptophyllum pictum* L.) and the *S. column. gold* and *C. albicans* colonies are growth indicators. The growth of *S. aureus* and *C. albicans* were measured at incubation time 24, 48, and 72 hours, which were then analyzed by analysis with Anava's one-way statistical test and continued with Duncan's 1%. The research found that the purple leaves ethanol extract (*Graptophyllum pictum* L.) has the growth inhibition of *Staphylococcus aureus* and *Candida albicans*, which is indicated by the comparison results of the optimal concentrations of *S. aureus* and *C. albicans*, were 50% and 90%, so that the purple leaf ethanol extract could recommended as an antibacterial.

Keyword: *Candida albicans*, purple leaves (*Graptophyllum pictum* L.), *Staphylococcus aureus*

INTRODUCTION

Microbes are closely related to everyday life. Microorganisms have the main characteristic that distinguishes them from one another, namely the organization of their cellular material (Waluyo, 2016). Microorganisms consist of bacteria, viruses, yeasts, molds, and protozoa, of all these types of microorganisms, one of which can be beneficial and can be harmful. Beneficial microorganisms are a group of normal flora, while harmful microorganisms have a tendency to cause various diseases or are called pathogenic microbes. Pathogenic microorganisms that cause infection in humans, one of which is

Staphylococcus aureus and *Candida albicans* (Anggraini et al., 2019). *S. aureus* is a normal flora found on human skin and human mucous membranes (Triana, 2014), while *C. albicans* is a fungal flora commonly found in the oral cavity, respiratory tract, digestive tract and vagina. *S. aureus* *C. albicans* which can trigger pathogenic which in excess can cause inflammation or abscess and cause infection, as well as infections. Control of infections caused by microbes is generally with the use of antibiotics. Inappropriate use of antibiotics causes resistance. (Retnaningsih et al., 2019) said that one way to minimize the

potential for antibiotic resistance to emerge is to use medicinal plants (back to nature). More than 30,000 plant species in Indonesia's forest areas are plants that have the potential as medicine (Fuadi, 2019). However, most people do not know the types of medicinal plants, so they are considered more as wild plants (Yuda et al., 2019). Plant species tend to be considered as wild plants, but have potential as medicinal plants, namely Purple Leaves (*Graptophyllum pictum* L.). Purple leaf plants are considered a group of shrubs, and are only considered as wild plants, so they tend to be ignored. The results of the study (Retnaningsih et al., 2019) showed that Purple leaves were effective against various infections, due to secondary metabolites contained in Purple leaves. The chemical components contained in purple leaves are alkaloids, nontoxic, flavonoids, glycosides, steroids, phenols, polyphenols, saponins, and tannins. The potential of purple leaves has not been widely known by the public, due to the lack of scientific documentation of the potential of purple leaves. The lack of information and public knowledge about the benefits of purple leaves in a sustainable manner indicates the need for literature on the benefits of purple leaves. Purple leaves extract was declared effective as an antibacterial of *S. aureus*. The final findings of this study are expected to be the scientifically documented potential of purple leaf ethanol extract as an antibacterial.

MATERIALS AND METHODS

This research is a comparative laboratory experimental study, with the aim at knowing the comparison of the potential of the purple leaf ethanol extract against *S. aureus* and *C. albicans*. The results measurement was carried out after the research treatment. The study was conducted at the Microbiology Laboratory of the State Islamic Institute of Palangka Raya, Central Kalimantan. The indicator of microbial growth inhibition

was measured based on the growth inhibition zone which was marked by a clear zone formed between *S. aureus* colonies on Nutrient Agar (NA) plate medium and *C. albicans* colonies on Sabouraud Dextrose Agar (SDA) plate medium with the outer side of the paper disc containing Purple leaf ethanol extract. Observations on the growth of *S. aureus* and *C. albicans* were carried out at incubation periods of 24, 48, and 72 hours after treatment.

Purple Leaf Extract Preparation

The stages in the preparation of the purple leaf extract begin with preparing the leaves to be used, which have been washed clean, then roughly cut to a size of ± 2 cm. The cut leaves are dried by sun tanning them. After the leaves dry, the next step is to blend the leaves until they have a smooth texture. The samples were dried in a blender until they became simplicia. Simplicia was immersed in ethanol for 3 days at room temperature.

The next step is a mechanical filtering process using a cloth, by squeezing the cloth. Filtering was carried out 3 times, namely 2 times filtering using cloth, and 1 time filtering using filter paper placed on a separating funnel.

Purple Leaf Extract Maceration

Extraction by maceration method of Purple Maserate leaves is then filtered, the filtrate is separated and the dregs are soaked back into new ethanol, the maceration is repeated ± 3 times until clear maserate is obtained. The obtained filtrate is concentrated in a rotary evaporator (40oC) or at a boiling temperature, until a thick extract or paste is obtained. The paste was then put into a vial and dried in a desiccator to obtain a dry extract.

The next step is to dilute the extract. Extract dilution was carried out with reference to the research design that had been previously designed, which included several concentrations of 40% (3.8 g of Purple leaf extract + 5.7 ml of sterile distilled water), 50% (4.75gr + 3.8 ml), 60 % (5.7 gr+2.85 ml), 70% (6.65gr+2.9 ml),

Friska *et al*, 80% (7.6 gr+0.951 ml), 90% (8.55 gr+0.951 ml). The diluted extract will be used in the analysis of the potential of Purple leaves against *S. aureus* and *C. albicans*. The extract obtained was stored in a vial bottle at a refrigerator temperature.

Microbial Culture

Bacterial cultures of *S. aureus* and *C. albicans* were obtained from the Microbiology Laboratory of the Biology-MIPA Tadris Study Program, IAIN Palangka Raya. The cultures were inoculated in liquid medium and then incubated at 37°C for 18-24 hours. Then standardized with the Mc Farland standard, with the aim that it is equivalent to 10⁸ CFU/ml by adding 0.85% NaCl.

Culture Stock Preparation

The stage of preparation of the parent stock of microbial culture begins with preparing nutrient both medium and inoculating microbial colonies from culture into the medium as much as 1 ose aseptically (Waluyo, 2016). Medium Nutrient both that had been inoculated with *S. aureus* and *C. albicans* colonies were incubated for 48 hours at 37°C. The incubation procedure aims at optimizing microbial growth (Aulia *et al.*, 2011).

Potential Analysis of Purple Leaf (*Graptophyllum pictum* L.) Against *Staphylococcus aureus* and *Candida albicans*

Antimicrobial analysis of *S. aureus* and *C. albicans* was previously carried out by culturing *S. aureus* and *C. albicans* in liquid medium and incubating for 24 hours. The treatment phase of the study was carried out by preparing nutrient agar (NA) and Sabouraud Dextrose Agar (SDA) plates as medium.

Both types of medium were dissolved in aquadest, and homogenized on a hot plate stirrer. Then 15 ml of the medium was put in a petri dish and wet sterilized using an autoclave at 121°C with a pressure of 15 lbs for 15 minutes (Anggraini *et al.*, 2019). The prepared medium was then used for the cultivation of *S. aureus* and *C. albicans* microbes. Liquid cultures of *S. aureus* and *C.*

albicans that have been incubated are gently shaken for 3 minutes, with the aim at spreading the microbial colonies evenly. Liquid cultures of *S. aureus* and *C. albicans* are planted as much as 0.5 ml in each medium evenly. Furthermore, the prepared paper discs were immersed at each concentration according to the research treatment, namely 40%, 50%, 60%, 70%, 80%, 90%. Soaking is done for 1 minute. The entire paper disc that has been soaked in Purple leaf extract is then placed in the middle of the surface of the NA & SDA plate aseptically using the disc method. The treated medium was incubated at 37°C. Data were collected at incubation periods of 24, 48, and 72 hours.

Growth Inhibition Zone Measurement

The results were read after being incubated for 24 hours at 37°C by measuring the zone of inhibition, namely the clear area around the discs where there was no bacterial colony growth. Measurement of *S. aureus* growth was based on the measurement of the diameter of the clear zone between the extract and the outer side of the clear zone, where the clear zone was a parameter of growth inhibition.

RESULTS AND DISCUSSION

The research data included the growth inhibition zone of *S. aureus* and *C. albicans*. Data were collected when the cultures of *S. aureus* and *C. albicans* were 24, 48, and 72 hours old at a temperature that had been set at 37°C.

Data analysis

Observation data was carried out after the treatment, namely at the incubation period of 24, 48, and 72 hours. The analysis of the observational data was then carried out by analyzing the one-way Anova statistical test and continued with the Duncan 1% test.

Treatment Data of Purple Leaf Ethanol Extract (*Graptophyllum pictum* L.) against *Staphylococcus aureus*

The data from the inhibition zone for the growth of *S. aureus* bacteria on Nutrient Agar (NA) basic medium are

Table 1 Growth Inhibition Zone of *Staphylococcus aureus* on NA Medium

Treatment	Average Inhibition Zone (mm)						
	24 Hours		48 Hours		72 Hours		
<i>Chloramfenicol</i> 0.1% (+)	3.52	b	3.59	b	4.04	b	
Aquades (-)	0.00	a	0.00	a	0.00	a	
Concentration	40%	4.04	b	4.37	bc	6.18	bcd
	50%	6.24	c	7.64	d	9.02	d
	60%	8.64	c	8.15	d	8.49	d
	70%	3.54	b	4.47	bc	4.76	bc
	80%	3.14	b	5.27	bcd	5.61	bcd
	90%	8.24	c	6.94	cd	7.19	cd

Notes:

Different notations in the same column show a significant difference based on the 1% DMRT test

The recapitulation data in Table 1 above has a varied mean zone of inhibition, where Chloramphenicol 0.1% as the positive control of the study had a lower mean zone of inhibition compared to the zone mean of the purple leaf ethanol extract. The use of Chloramphenicol as a positive control research with consideration of its potential to be bacteriostatic or inhibit bacterial growth, is equivalent to the potential of Purple leaf extract which is known to contain many potential compounds as bacteriostatic. This chemical mechanism will result in a certain synergistic effect after treatment, because compounds that are bactericidal if combined with bacteriostatic compounds, the bacteriostatic effect will stop the growth of bacterial cells. Chloramphenicol is one of a group of antibiotics that can inhibit microbial cell protein synthesis.

The mechanism of growth inhibition was observed at 24 hours of observation, 3.52 mm (*Chloramfenicol* 0.1%). Meanwhile, the mean zone of purple leaf extract at 24 hours of observation was 6.24 mm. This shows that the inhibitory power of the extract on the growth of *S. aureus* at a certain concentration is stronger than the positive control of the study. At a certain concentration, the antibacterial power of Purple leaf extract is stronger in inhibiting the synthesis of peptidoglycan in bacterial cell walls,

compared to 0.1% Chloramphenicol, where it is known that these components are important components to maintain the integrity of bacterial cell walls, while humans do not need peptidoglycan to maintain cell wall integrity. .

However, in a higher concentration, Chloramphenicol is one type of antibiotic that has a broad spectrum of activity, namely an antibiotic that can kill various kinds of bacteria including normal flora bacteria, so that 0.1% Chloramphenicol is quite representative used as a comparison in this study. .

The observed data in Table 1 is confirmed by Duncan's test results of 1% at 24-hour observation, namely the smallest concentration which has almost the same inhibitory power as the higher concentration is 50% concentration, so that 50% concentration is interpreted as the effective concentration of the study. The 48-hour observation above showed that the treatment of purple leaf ethanol extract (*Graptophyllum pictum* L.) had a significant effect on *S. aureus*. This is evidenced by the results of the average inhibition zone Chloramphenicol is 3.59 mm, while the extract is 7.64 mm. The treatment in the form of administration of purple leaf ethanol extract had almost the same effect as 24-hour observation, namely the 50% concentration was not significantly different from the 90% concentration, so the effective

concentration, namely the 50% concentration, was interpreted as the effective concentration in inhibiting the growth of *S. aureus*. This is because Purple leaf extract contains alkaloids that function as antibacterial. This statement is in line with research (Suryaku, 2017) which states that alkaloids have the ability as antibacterial by denaturing proteins and damaging cell membranes.

Damage to cell membranes results in changes in cell permeability, resulting in growth inhibition. The process of growth inhibition due to the content of secondary metabolites is characterized by the formation of an inhibition zone between the paper disc and microbial colonies. This inhibited cell growth, in a long time will cause cell death. This can be seen during the 24 hour incubation period, which is interpreted as the antimicrobial content in the paper disc is directly proportional to the greater the mean zone of microbial growth inhibition.

Flavonoids in secondary metabolites of plants also function as antibacterial, namely forming complex compounds with extracellular and dissolved proteins so that they can damage bacterial cell membranes followed by the release of intracellular compounds. Intracellular compounds that are pulled out of the cell result in the rupture of the membrane or plasma membrane, which in a certain incubation time results in cell death (Hujjatusnaini *et al.*, 2021).

In vitro cell death is defined as a process of necrosis, where cell or tissue death occurs as a result of an irreversible degeneration process, while the process between degeneration and cell death is also called necrobiosis. The occurrence of necrosis is estimated to be observed about 6 to 8 hours after cell death. Macroscopically, the cells or tissues that undergo necrosis are characterized by paleness, followed by the condition of the tissues becoming softer and there appears to be a demarcation (limiting) with healthy tissue. This cell or tissue barrier is used as a cell death zone. Usually around cells or tissues that

undergo necrosis are always accompanied by inflammatory cells, because dead cells are strange objects to the body.

Observations continued at 72 hours incubation period showed a very significant decrease in inhibition at 72 hours incubation period, the notation showed higher concentrations (90%) which did not differ statistically with lower concentrations (60%). The data illustrates that the 50% concentration still has the same ability with the incubation period of 24 hours and 48 hours. It can be interpreted that the concentration of 50% is an effective concentration in inhibiting the growth of *S. aureus* bacteria.

(Hujjatusnaini *et al.*, 2021) explained that the growth of *S. aureus* was almost the same as the growth of other normal flora. because basically *S. aureus* is a bacterium that includes normal flora, especially on the skin and mucous membranes in humans. *S. aureus* has an optimum temperature at 35oC, where the limit of growth temperature is between 15oC and 40oC. Another tendency is that these bacteria prefer aerobic conditions, and these conditions are very good for the growth of *S. aureus* bacteria. *S. aureus* can grow in air containing only hydrogen and Optimum pH 7.4 for growth. *S. aureus* is a facultative anaerobe. Young *S. aureus* colonies were colorless, but their growth was formed by pigments that were soluble in alcohol, ether, chloroform, and benzoyl.

S. aureus is a normal flora found on the skin, respiratory tract and digestive tract of food in humans. *S. aureus* has invasive properties, can cause hemolysis, form coagulation, liquefy gelatin, and ferment mannitol. *S. aureus* can cause disease because it has the ability to multiply and spread widely in tissues and through the formation of extracellular substances. As long as *S. aureus* continues to increase in the tissue, leukocytes will also increase to kill these bacteria.

Based on the data above, it can be seen that the 50% concentration of the

purple leaf ethanol extract was effective and able to inhibit the growth of *S. aureus*. This is because purple leaves contain secondary metabolites that function as antibacterial. This is because Purple leaves have secondary metabolites in the form of flavonoids and alkaloids. In line with research (Aulia *et al.*, 2011) which says that Purple leaves can be used as antibacterial.

Purple leaf ethanol extract contains secondary metabolites in the form of flavonoids and alkaloids that have benefits as an inhibitor of microorganisms, one of which is *S. aureus* bacteria. Research results from (Retnaningsih *et al.*, 2019) confirm that Purple leaves contain secondary metabolites such as alkaloids, flavonoids, saponins, steroids and tannins. The results of this study confirmed that one of the ingredients possessed by Purple leaves such as flavonoids and alkaloids was proven to be antibacterial. Flavonoids are included in phenolic compounds found in nature which have many properties, one of which is antioxidant and antibacterial, so the findings of this study are in line with previous studies where Purple leaf extract was proven to have an inhibitory effect on the growth of *S. aureus*.

In addition to flavonoids, the active metabolite of alkaloids contained in Purple leaves is known to work as an antibacterial. The mechanism of action of alkaloid compounds is by interfering with the peptidoglycan constituent components in bacterial cells. Disruption of the peptidoglycan constituent of bacterial cells causes the cell wall layer to be damaged, resulting in cell death or necrosis.

Peptidoglycan is the main component of the bacterial cell wall, which is rigid. Because of its rigid composition, peptidoglycan functions as a part that is responsible for maintaining cell integrity, as well as being a determinant of cell shape. Peptidoglycan is only found in

certain bacterial species, one of which is *S. aureus*. However, the structure of peptidoglycan found in Gram negative bacteria is different from that of Gram positive bacteria. In general, this peptidoglycan (murein) is a polysaccharide consisting of two derived sugars, namely N-acetylglucosamine acid and N-acetyl muramic acid linked by -1,4 bonds, and a short peptide chain. Therefore, peptidoglycan has a direct involvement in the process of cell death.

Another potential antimicrobial activity is tannin which is predicted to cause a decrease in bacterial cell permeability and turgosity, so that the cell wall shrinks. The decrease in cell turgidity will disrupt the permeability of the cell itself and cause cell wall damage. Cell membrane permeability is the ability of the cell membrane to transport needed substances from the outside or the extracellular environment into the cell or cytosol (Hujatusnaini *et al.*, 2021).

Purple Leaf contains alkaloids, flavonoids, and tannins in dominant quantities, thus strengthening its potential as an antibacterial in the dominant composition. The mechanism of action of the disruption of cell wall permeability generally involves the role of terpenoid metabolites, because terpenoids also act as antibacterials. Terpenoids are thought to be involved in membrane damage of lipophilic compounds. Terpenoids are reactive to transmembrane proteins or porins in the extracellular membrane of bacterial cells. This interaction forms a strong polymeric bond, resulting in damage to the porin which ultimately results in a decrease in the permeability of the bacterial cell wall. If cell permeability is disturbed, the nutrients needed for bacterial cell growth are also disrupted. At a certain incubation period, bacterial cells will experience growth inhibition, marked by the formation of an inhibition zone *in vitro*. The mean of growth inhibition zone of *S. aureus* is presented in Figure 1.

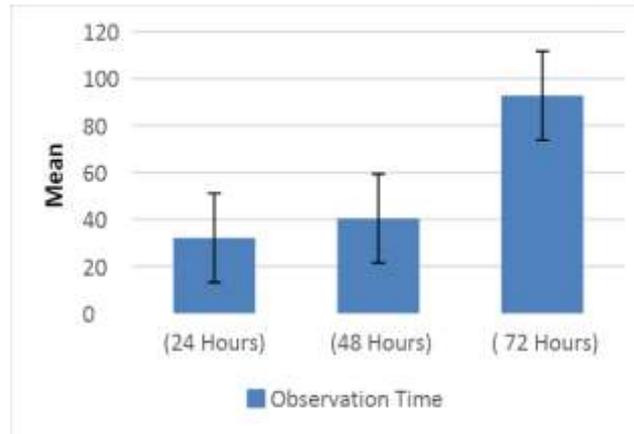


Figure 1. Mean of growth inhibition zone of *Staphylococcus aureus*

The mean value in Figure 1 illustrates that 72 hours of incubation has a greater effect on optimizing the effect than 24 hours and 48 hours, so the results can be used as indicators for determining the most effective concentration in the formulation. Observation of the growth inhibition zone of *S. aureus* was confirmed by the comparison of Duncan test results presented in Table 1.

Data on Treatment of Purple Leaf Ethanol Extract (*Graptophyllum pictum* L.) against *Candida albicans*

The data from the observation of the inhibition zone for the growth of *C. albicans* bacteria on SDA medium are presented in Table 2. The measurement of the growth of *C. albicans* is based on the results of the measurement of the diameter of the clear zone between the extract and the outer side of the clear zone, where the clear zone is a parameter of growth inhibition.

C. albicans is a dimorphic fungus because it has the ability to grow in two different forms, namely as shoot cells which will develop into blastospores and produce sprouts which will form pseudohyphae. The difference in this form depends on the external factors that influence it (Maulidah, 2019)

C. albicans is often found as a cause of infectious diseases of the female genitalia and perigenital area. *C. albicans*

is a normal flora group of fungi that live in the human body such as in the mouth, throat, vagina and digestive system. *C. albicans* in the human body are saprophytic and pathogenic (Itsa *et al.*, 2018).

C. albicans is microscopically round, oval, with a smooth surface, smooth or folded, yellowish white in color and has a yeast odor and has a complex cell wall structure, 100 to 400 nm thick. *C. albicans* can grow at a pH of 4.5-6.5 and at a temperature of 28° C-37° C (Nuryati & Huwaina, 2015). The disease caused by *C. albicans* is called candidiasis. Candidiasis is an acute and sub-acute fungal disease caused by *Candida* sp species, usually *C. albicans* (Farizal & Abdul Rahman Serbasa Dewa, 2017). This microorganism is one of the most potent groups of yeasts and is closely related to infections of the reproductive system. These microorganisms are saprophytic, so that under conditions of pH and humidity suitable for growth, they tend to be pathogenic and cause inflammation and infection.

Pathogenicity is the ability of microbes that are pathogenic, or cause infection or inflammation related to their ability to produce enzymes or toxins. This pathogenicity will increase along with the

Table 2. Growth Inhibition Zone of *Candida albicans* on SDA Medium

Treatment	Inhibition Zone Mean (mm)			
	24 hours	48 hours	72 hours	
<i>Albothyl</i> 0.25% (+)	8.44 b	8.81 b	26.44 d	
Aquades (-)	0.00 a	0.00 a	0.00 a	
concentration				
40%	13.74 c	14.79 c	15.14 bcd	
50%	5.13 b	7.88 b	11.34 bc	
60%	7.89 b	8.02 b	8.64 b	
70%	14.14 c	14.69 c	16.22 bcd	
80%	17.14 c	17.74 c	19.14 cd	
90%	12.14 c	14.94 c	16.04 bcd	

Notes:

Different notations in the same column show a significant difference based on the 1% DMRT test.

The recapitulation data in Table 2 has an average inhibition zone of 0.25% *Albothyl* as a positive control study has a lower mean zone of inhibition compared to the zone average of the purple leaf ethanol extract. (Mutiara Sandy & Burhanisa Irawan, 2019) describes *Albothyl* as an antiseptic and disinfectant for mucosal tissue infections containing policresulen compounds. *Albothyl* 0.25% was used as a positive control study with consideration of its role against infections caused by fungal and bacterial groups, where the condensation process of meta cresol sulfonic acid and methanol. Furthermore, it is known that policresulen is usually used to stop local bleeding or local hemostatic, because policresulen is a condensation product compound metacresol sulphonic acid and methane. The mechanism of action of these compounds will eventually cause the infected cells to die.

At the observation time of 24 hours, 8.44 mm (*Albothyl* 0.25%) Meanwhile, the mean zone of purple leaf extract at the observation time of 24 hours was 13.74 mm. This shows that the inhibitory power of the extract on the growth of *C. albicans* is stronger than the positive control of the study. The data were also confirmed with the Duncan test results 1% at 24 hours observation, it can be interpreted that the effective treatment level of purple leaf ethanol extract at 24 hours observation

time is 40% concentration. The results of the observation of the effective and optimum concentration of purple leaf extract during an incubation period of 24 hours are in line with the statement (Maya Ayuningtias, 2017) that the ethanolic extract of purple leaf has antifungal activity against *C. albicans*.

Observations at the 48-hour incubation period showed that the effective treatment level of the purple leaf ethanol extract at the 48-hour observation time was a concentration of 40%. The effective concentration at 48 hours of observation is not different from 24 hours, so it can be interpreted that the 40% concentration still has the same inhibitory power up to 48 hours.

This inhibitory power is influenced by the flavonoid content contained in the ethanol extract of purple leaves which can function as antifungal (Sari, 2017). The effectiveness of secondary metabolites in ethanol extract as antifungals was then observed for 72 hours, at 72 hours showed that *Albothyl* 0.25% had the largest inhibition zone. These data indicate that the effectiveness of secondary metabolites contained in the ethanolic extract of Purple leaves is only able to last up to 48 hours, while at 72 hours it has an inhibitory power that is not better than the antifungal at *Albothyl* 0.25%. The decrease in inhibition at 48 hours and decreased at 72 hours

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incubation was influenced by the weaker fungicidal properties in the extract. Its fungistatic properties are decreasing and can even disappear altogether.

The results of statistical analysis showed that the purple leaf ethanol extract had a significant effect on the Sig

value. $0.000 < 0.01$, both at incubation time of 24 hours, 48 hours, and 72 hours. However, when viewed from the comparison of the mean values (Figure 2), the incubation period of 72 hours has a higher mean value than other incubation times.

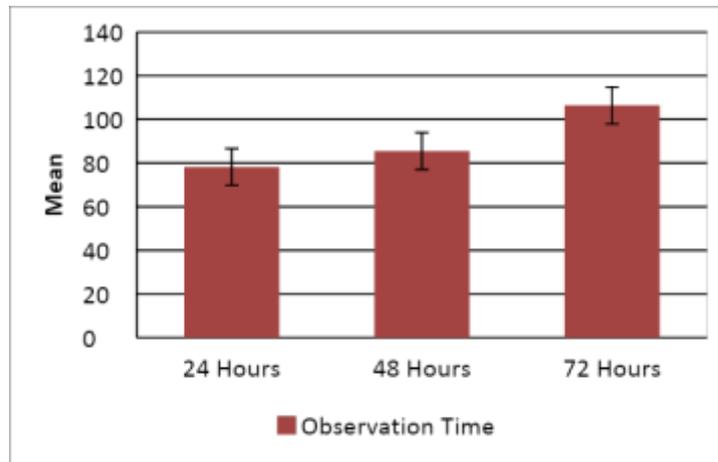


Figure 2. Mean of Growth Inhibition Zone of *Candida albicans*

The comparison of the mean value in Figure 2, where the comparison of the mean gives the fact that the incubation period of 72 hours has a much greater difference in effect than 24 hours and 48 hours, so the results can be used as an indicator for determining the most

effective concentration. To determine the potential tendency of purple leaf as an antimicrobial, it can be seen from the comparison of the inhibitory power of purple leaf against *S. aureus* and *C. albicans* as presented in Figure 3.

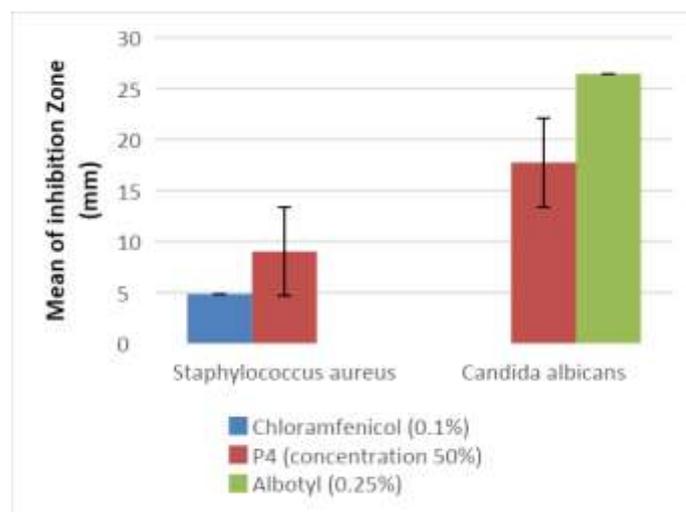


Figure 3 Comparison of formed inhibitory power from *Staphylococcus aureus* and *Candida albicans*

Figure 3 above provides an illustration of the comparison of inhibition formed by *S. aureus* and *C. albicans*. The inhibitory

power formed from *S. aureus* was 4.84 mm (Chloramfenicol 0.1%), for a 50% concentration of 9.02 mm. Meanwhile, the

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inhibition zone formed from *C. albicans*, 26.44 mm (Albothyl 0.25%), for a 50% concentration of 17.74 mm. If seen from the comparison of the image above, it can be interpreted that the ethanolic extract of purple leaf has a greater potential as an antifungal. This is based on the inhibition zone formed, which is 17.74 mm. In line with the statement (Sari, 2017) that the ethanol extract of Purple leaves has an antifungal effect on the growth of *C. albicans*. The ethanolic extract of purple leaves can inhibit the growth of *C. albicans* or has the potential as an antifungal because it contains secondary metabolites in the form of saponins. In line with research (Sari, 2017) that saponins have antifungal or antifungal effects. Saponins work by damaging the cytoplasmic membrane, so that it can inhibit the growth of *C. albicans*.

Cytoplasm is an intracellular fluid found between the plasma membrane and the nucleus, where chemically it is known that about 70-90% of the cytoplasm consists of water and solid components (proteins, carbohydrates, lipids, and inorganic substances). The cytoplasmic membrane is the membrane that surrounds the cytoplasm. This membrane is composed of a layer of phospholipids and proteins. This cytoplasmic membrane is a membrane that functions to enclose the cytoplasm and its contents, located just below the cell wall but not tightly bound to the cell wall. Structurally, it is understood that this membrane is very important for cells, because it acts as a separator between the inside of the cell and the environment. In connection with the mechanism of action of saponins in destroying the cytoplasmic membrane, it is closely related to the destruction of the permeability and selectivity of the membrane, so that the ability of the membrane to maintain cell osmotic pressure is disrupted.

Saponins are antifungal compounds that have an energy mechanism that can interfere with electron transfer. This electron transfer that occurs will disrupt

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the stability of phosphorylation in the mitochondria, as a result, energy metabolism in the mitochondria is disrupted. This cellular condition will cause oxygen intake to decrease in mitochondria, so that the function of the tricarboxylic acid cycle was disrupted. The antifungal mechanism of the content of secondary metabolites in Purple leaves results in disruption of the formation of Adenosine Triphosphate (ATP) and Adenosine Diphosphate (ADP).

The inhibition zone formed can also be interpreted as the occurrence of protein denaturation of microbial cells. Denaturation of cell proteins causes shrinkage of cell walls, which in the longer term will result in necrosis. The content of metabolites that act as antifungals will generally diffuse to the fungal cell membrane, which then interferes with cellular metabolic pathways (Maya Ayuningtias, 2017).

Another antifungal mechanism according to (Hujjatusnaini *et al.*, 2021) is an antifungal mechanism that suppresses the growth of fungal hyphae and mycelium, thereby inhibiting fungal growth. Flavonoid compounds enter into fungal cells through holes in the cell membrane that are formed because phenolic compounds have denatured cell membrane lipids. These protein compounds will be denatured by flavonoids through hydrogen bonds. The ability of flavonoids to bind to proteins causes inhibition of cell wall formation, so that hyphae growth is also inhibited because the required cell wall composition is not fulfilled.

In addition, other mechanisms were also described, including the involvement of cytochrome P450 enzymes needed to maintain the integrity of fungal cell membranes. Cytochrome P450 (CYP) is a group of enzymes or isoenzymes that are known to have a very important role in metabolic processes, namely helping to change the content or efficacy of drugs in an ingredient into active metabolites. The antifungal metabolism is able to reduce the surface tension of the sterol

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membrane of the cell wall of *C. albicans*, resulting in an increase in its permeability. This sterol membrane is a membrane that contains sterol fat, namely ergosterol which is only found in the group of fungi, whose function is the same as cholesterol in animals. The increase in permeability causes the intracellular fluid to become more concentrated and is drawn out of the cells, resulting in swelling of the cells, and within a certain incubation time causes fungal cell death.

Fungal cell walls contain mannoproteins, chitin and alpha, and beta-glucans which play an important role in protection, maintaining cell morphology and cell rigidity, metabolism, ion exchange and filtration, antigenic expression. The antifungal metabolite compounds in purple leaves will inhibit the synthesis of -glucan fungal cell walls, some of which are a group of fungi including *Candida albicans* and *Candida glabrata* (Apsari & Adiguna, 2013).

Several other antifungal compounds are known to interfere with energy metabolism in mitochondria, namely in the process of electron transfer and phosphorylation. These antifungals interfere with the chemical process of electron transfer, which begins with inhibition of energy metabolism in the mitochondria. The inhibition of electron transfer reduces oxygen and interferes with the functioning of the tricarboxylic acid cycle. If electron transfer is inhibited, further chemical processes will not occur. With the inhibition of electron transfer, the phosphorylation step will not occur. As a result, the formation of adenosine triphosphate (ATP) and adenosine diphosphate (ADP) is also inhibited.

The antifungal mechanism of action includes phenol groups which will form complexes with proteins in the cell membrane, resulting in clumping. The clumped proteins are denatured. This denaturation is characterized by the process of breaking hydrogen bonds, hydrophobic interactions, salt bonds and the formation of molecular folds, protein denaturation, resulting in decreased cell

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membrane permeability, disrupted nutrient transport in cells, and ultimately inhibited fungal growth. This growth inhibition process appears as a zone of inhibition of cell growth in vitro.

CONCLUSION

Purple leaf ethanol extract (*Graptophyllum pictum* L.) had a significant effect at the 1% significance level. The ethanolic extract of purple leaves was proven to have the growth inhibition of *S. aureus* and *C. albicans*. However, purple leaf ethanol extract has more potential as antifungal.

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