# POTENTIAL OF ENDOPHITE FUNGI ISOLATE FROM LEAF OF CHINESE TEMPERATURE (Cassia alata L) IN INHIBITING THE GROWTH OF THE FUNGI Malassezia furfur

Rifka Sadapu1), Syam S Kumaji 2), Adnan Malaha 3) <sup>1,3)</sup>BinaMandiri University Gorontalo <sup>2)</sup>Gorontalo State University E-mail: rifkasadapu07@gmail.com

## ABSTRACT

This study aims to determine the effect of endophytic fungal isolates from the leaves of the Chinese Ketepeng (Cassia alata L) plant on the growth of Malessezia furfur and to determine the significant differences of each treatment of endophytic fungal isolates from the leaves of the Chinese Ketepeng (Cassia alata L) plant on the growth of Malessezia furfur.

The method in this study used a completely randomized design (CRD) with 4 treatments and 6 replications. The treatments were: JEKA 1 (Chinese KetepengEndophytic Fungus 1), JEKA 2 (Chinese KetepengEndophytic Fungus 2), Positive Control (Ketoconazole), and Negative Control (Aquadest).

The results showed that 2 isolates of endophytic fungi were successfully isolated from the leaves of the Chinese Ketepeng plant. After testing the inhibitory power against the Malassezi furfur fungus, the JEKA 1 isolate obtained an inhibition zone value of 10 mm and the JEKA 2 isolate an inhibitory zone value of 11 mm.

The results of the analysis using the Kruskal-Walis test showed that there was an effect of endophytic fungal isolates from the leaves of the Chinese ketepeng plant (Cassia alata L) on the growth of the fungus Malassezia furfur. Then from the results of further tests using Duncan's test, it was found that each treatment, namely the negative control treatment was significantly different from JEKA 1, JEKA 2 and positive control, while there was no significant difference between JEKA 1, JEKA 2 and positive control.

Keywords : Endophytic Fungus, Chinese Ketepeng, Malassezia furfur

#### PRELIMINARY

Indonesia is one of the countries that has biological resources which ranks second after Brazil, one of which is the diversity of medicinal plant species. But there are still many people who do not know the efficacy of the types of growth that have the potential as medicine. One of the plants that can be used as a medicinal plant is the Chinese Ketepeng plant Alata following (Cassia L.) the morphology of the Chinese Ketepeng plant which consists of leaves, flowers, and fruit.

The leaves of the Chinese Ketepeng (Cassia alata L) are oblong to oval breech, are even pinnate compound leaves in pairs of 5-12 rows, have stiff leaflets 5-15 cm long, 2.5-9 cm wide, The tip of the leaf is blunt with a pointed leaf base and the leaf edge is flat. The leaf veins are pinnate with short leaf stalks  $\pm$  2 cm long and green.

The Chinese ketepeng flower (Cassia alata L) is a compound flower arranged in long and erect stems located at the ends of its branches with bright yellow flower crowns.

The Chinese Ketepeng fruit also has wings on both sides with a length of 10-20 mm and a width of 12-15 mm. If the fruit is ripe, then on both sides it will open or break so that the seeds contained in the pod will be thrown out. Seeds owned by Chinese ketepeng (Cassia alata L) are triangular in shape and flattened, totaling 50-70 seeds in each pod.

Chinese ketepeng (Cassia alata L) is one of the plants that can be used as traditional medicine to treat tineaversicolor, ringworm, ringworm, intestinal worms, constipation, and canker sores. This is due to the chemical content contained in it. According to [9] that the Chinese ketepeng leaf contains several chemical compounds, namely alkaloids, tannins, chrysofanic acid, glycosides, aloeemodina, bitter substances, tanning substances, and flavonoids.

Furthermore, [7] said that the leaves of Chinese Ketepeng (Cassia alata L.) can be used as traditional medicine due to the chemical content contained in it such as emodina. rein aloe rein aloe chrysophanic emodinadiantron, acid (dehydroxymethylantroquinone), tannins, alkaloids, and flavonoids. Flavonoids in anti-inflammatory, have plants antiallergic, antimicrobial, antioxidant effects, and are effective against several groups of fungi and bacteria. The activity of Ketepeng extract as an antibacterial has been proven by several research results.

Research conducted by [9] that ethanol extract is able to inhibit the growth of bacteria that causes thrush which is characterized by a reduction in the number colonies at different of extract concentrations. Furthermore, [16] said that at a concentration of 2.5% Chinese ketepeng leaf extract was better at inhibiting the growth of the diameter of the fungus Phytophthorapalmivora which was 30.18% and after being analyzed using the linear regression equation method, the concentration of 2.5% had the largest y value, which was 30.,48%.

The need for large amounts of antimicrobials in tackling pathogenic microbes encourages researchers to make new antimicrobial discoveries. For example, using endophytic microbes that are in symbiosis with plants, one of which is endophytic fungi. According to [21] thatendophytic fungi are organisms that live in plant tissues such as roots, stems, leaves, flowers and fruits.

Endophytic fungi and host plants can be symbiotic mutualism, in this case the endophytes get nutrients from the metabolism of plants that have activity for nutrients and active compounds needed during their life. The ability of endophytic microbes to produce secondary metabolites according to their host plants is a very large and reliable opportunity to produce secondary metabolites from endophytic microbes isolated from their host plants.

Endophytic fungi can live in plant tissues both in roots, leaves, stems, bark and even in plant reproductive structures and can provide tolerance to biotic and abiotic stress. Each plant has one or more species of endophytic fungi, and it has been observed that there are three types of beneficial interactions between endophytic fungi and their host plants namely increased growth of the host plant, increased resistance of the host plant to biotic and abiotic stresses, and accumulation of secondary metabolites, including bioactive compounds used as medicine, which was originally produced by plants.

Endophytic fungi can produce secondary metabolites according to the host plant, which is an opportunity to produce secondary metabolites from the host plant. If endophytic fungi isolated from a medicinal plant can produce alkaloids or secondary metabolites similar to the original plant or even in higher amounts, then we do not need to cut down the original plant to be taken as simplicia which will likely take decades to harvest.

Endophytic microorganisms will release a secondary metabolite which is an antibiotic compound itself. Secondary metabolites are compounds synthesized by microbes, not to fulfill their primary needs (grow and develop) but to maintain their existence in interacting with their environment.

Secondary metabolites produced by endophytic microorganisms are antibiotic compounds that are able to protect plants from insect pests, pathogenic microbes, or their predatory animals, so that they can be used as biocontrol agents.

Endophytic fungi are able to produce bioactive compounds, such as antibacterial, anticancer, antiviral &antifungal compounds [5]. Endophytic fungi synergize with their host plants through a symbiotic relationship of mutualism. Endophytic fungi function to overcome the problem of antibiotic resistance and treat diseases in humans, plants and animals. According to [8] that endophytic fungi are able to inhibit the growth of pathogenic fungi. One example of a pathogenic fungus is Malassezia furfur.

*Malasseziafurfur*is a skin fungus that infects people with tineaversicolor. This fungus causes the formation of very fine scales without any other symptoms or coarser scales accompanied by a rash that feels itchy and causes pain between the fingers.

*Malasseziafurfur*have yeast cells oval, unicellular or globular in shape with buds (4-8 m) and short, septate and sometimes branched hyphae (2.5-4 m in diameter & variable length). Malassezia furfur forms a yeast, dry and white to cream in color. On the skin of fungal sufferers appear as round spores and short hyphae. The macroconidia are in the form of lines that have different refractive indices from their surroundings and certain distances are separated by partitions or beads such as necklaces, hyphae appear short, straight or bent with many small grains that are clustered.

This disease is found throughout the world, especially in hot climates, so this disease is cosmopolitan. In Indonesia, panu is a superficial mycoses with a high frequency. Transmission of tineaversicolor occurs when there is contact with fungi that cause other triggers, namely the frequent use of accessories that fit the skin, such as watches, jewelry, socks, and shoes. Therefore, the personal hygiene factor is very important. In fact, there are people who are susceptible to infection and some are not. So besides personal hygiene, there are other factors that influence the occurrence of infection.

Humans get the infection when the cells of the fungus Malassezia furfur attach to the skin. The lesions begin as small, thin patches which then become numerous and spread, accompanied by the presence of scales. Skin disorders in people with tineaversicolor are obvious, because in people who have black skin, phlegm is a spot with hypopigmentation, while in people with white skin, it is as a spot with hyper pigmentation. Thus the color of this skin disorder can vary (versicolor). These skin disorders are mainly on the upper body (neck, face, arms, chest, abdomen, etc.), in the form of small round patches (nummular), or even wide like plaques on the lungs that are chronic. Usually there are no complaints, there is itching when sweating, there is a feeling of shame with cosmetic reasons.

Early fungal infection appears as yeast cells (saprophytes) and after yeast cells become mycelium (hyphae) they will turn into pathogens, causing lesions on the skin. As a result of increased fungal growth resulting in fungal colonization on the skin. It is often associated with certain factors, such as oily skin, prematurity, prolonged antimicrobial treatment, corticosteroids, extracellular glycogen accumulation, chronic infection, excessive sweating, use of skin lubricants and sometimes pregnancy.

Fungus can cause the feet to become cracked, resulting in a bacterial infection. Fungal infections that usually appear on the feet during hot/warm weather. The purpose of this study was to determine the significant differences between treatments of endophytic fungal isolates from the leaves of the Chinese Ketepeng (Cassia alata L) plant in inhibiting the growth of Malessezia furfur.

The benefit of this research is to provide information to the public that the Chinese Ketepng plant has health benefits in terms of being a traditional medicine. Provide information about the presence of endophytic fungi in Chinese ketepeng (Cassia alata L). which has potential as a producer of antifungal compounds. As an ingredient in the development of microbiology and phytochemistry courses.

Based on the description above, the authors are interested in conducting a research entitled The Potential of Endophytic Fungus Isolates from the Leaves of the Chinese Ketepeng Plant (Cassia alata L) in inhibiting the growth of Malassezia furfur.

#### **RESEARCH METHODS**

This research is a quantitative research with the type of laboratory experimental research. This study used a laboratory experimental method, with a completely randomized design (CRD) consisting of 4 treatments and 6 replications.

The data collection techniques used in this study are the tools used in the research are autoclave, stir bar, bunsen, petri dish, erlenmeyer, measuring cup, beaker. scissors, incubator. caliper. catheter, laminar air flow cabinet (LAFC), micropipette, oven, ose, bath, tweezers, test tube rack, incubator shaker, and test tube. The materials used in this study included Chinese ketepeng leaves (Cassia alata L), aluminum foil, filter paper, aquades, potato dextrose agar (PDA) media, potato dextrose broth (PDB) media, 5% NaOCl, 70% alcohol. , and Malassezia furfur.

Research preparation begins with the sterilization of tools and materials to be used such as stirring rods, petri dishes, erlenmeyer, measuring cups, beakers, scissors, caliper, catheters, loops, tweezers, test tube racks, test tubes. washed with soap containing antiseptic ingredients first and then dried. After drying, the tools were wrapped in aluminum foil, then put in the oven at 175°C for 2 hours. Meanwhile, materials

such as PDA and PDB media were put into an autoclave at 121°C with a pressure of 15 psi (per square inch) for 15 minutes.

Leaf Extract of Chinese Ketepeng (Cassia Alata L) The sample used was the Chinese Ketepeng plant (Cassia Alata L) which was obtained from the Boalemo Regency, Gorontalo Province, the part taken was the fresh leaf. Sampling was carried out in the morning, the leaves determined as samples were located on the branches of one third of the plant from the top, namely the final shoots (leaves 3-4) and the previous shoots (leaves 5-6) which were physiologically perfect.

Making the media, Making Potato Dextrose Agar (PDA), weighing the PDA according to the provisions on the packaging and 5 petri dishes, obtained as much as 2.9gr of PDA then dissolved in 75 ml of distilled water and then put into an erlenmeyer and covered with cotton and aluminum foil. Then homogenized with the help of a bath to boil. After that, it was sterilized in an autoclave for 15 minutes at 121oC. The sterile media was poured into a petri dish aseptically and cultured at room temperature.5 x  $15\frac{39}{1000}$ 

While the manufacture of Potato Dextrose Broth (PDB), PDB is weighed based on the provisions on the packaging and 2 75ml Erlemeyer, 5 obtained GDP of 1.98gr then dissolved in 75 ml of distilled water then put into Erlenmeyer and covered with cotton and aluminum foil, then homogenized with the help of heater until it boils. After being homogenized, put it in the Erlemeyer closed using cotton and aluminum foil. Then sterilized in an minutes autoclave for 15 at 121oC. x  $15\frac{26,5}{1000}$ 

Surface Sterilization of Chinese Ketepeng Leaves (Cassia alata L), the fresh leaves were washed using running water until clean. Furthermore, surface sterilization is carried out which is carried out in a Laminar Air Flow Cabinet

(LAFC). The leaves were then immersed in 5.3% NaOCl solution for 1 minute. After that it was dipped in alcohol for 30 seconds then continued rinsing using sterile distilled water as much as 100 L for 1 minute then removed and dried on a dry tissue. This stage aims to remove particles or microorganisms that are not needed [13].

Isolation and Purification of Endophytic Fungus, leaves that have been previously sterilized both sides of the leaves are sliced with a size of  $\pm$  1x1 cm with a sterile catheter in a Laminar Air Flow Cabinet (LAF) and then planted with the inner tissue of the leaves touching the surface of the PDA media carefully. Each petri dish contains five leaf slices and then incubated at 37oC for 5-7 days. After incubation, endophytic fungi will grow around the leaves [13].

The growing endophytic fungi will be used as samples. Samples of endophytic fungi growing on PDA media were subcultured to PDA media and then incubated at 37°C for 5-7 days until pure were obtained. Furthermore, colonies samples of pure endophytic fungus isolates contained in PDA media were grown into an Erlemeyer with liquid media media, namely PDB which functions to ferment endophytic fungi. After that it was incubated for 12 days. This aims to obtain the metabolite compounds produced by endophytic fungi [13].

Observation of morphology of endophytic fungi, isolates of endophytic fungi obtained were then observed macroscopically and microscopically. For macroscopic observations, the morphology includes the shape of the colony, the shape of the edge of the colony, the upper surface of the colony and the color of the colony. While microscopic observations were carried out magnifying using a glass and а microscope.

Jurnal Ilmiah Dr. Aloei Saboe (JIAS) 4059

Secondary Metabolite Collection, samples of pure endophytic fungal isolates grown in PDB media were previously poured into tubes and then centrifuged at 10,000 rpm for 20 minutes. So that the antimicrobial activity of the supernatant culture (metabolite compounds) will be separated from the broth [17]. After that, the supernatant is taken for further processing.

Rejuvenation and Confirmation of Malassezia furfur, before being used for testing, this test fungus was rejuvenated in PDA growth media. Colonies that grew from the results of the rejuvenation were used as test mushrooms. After that, one fungal colony was taken using an ose needle and then inoculated on the PDB medium in the Erlemeyer. After that, incubate in a shaker incubator at 37°C for 1x24 hours. This rejuvenation was carried out because in testing the effectiveness of antifungals, fresh fungal colonies aged 24 hours were needed [17].

Preparation of Test mushroom suspension, the test mushroom which has been rejuvenated in PDB media is made a suspension using a spectrometer, the wavelength used is 580 nm then diluted to obtain an OD of 0.6 or equivalent to 800x106 cFu/ml, if appropriate, this suspension will be used as a test mushroom.

Antifungal Activity Testing, effectiveness test using pour plate method. The pour plate method is a method in which the suspension of the test fungus is first put into a petri dish, then PDA media is added and then mixed and allowed to solidify. Furthermore, the discs that had been soaked for 30 minutes in the supernatant and control were placed on the surface of the PDA media. After that, it was incubated at 37°C for 1x24 hours. After incubation. observations were continued by looking at the inhibition zone. The scheme for laying disc paper on the test media is presented in (Figure 1).



Figure 1 Schematic of laying disc paper on the test media

Inhibition zone measurements, observations were made after 24 hours of incubation. The inhibition zone formed around the disc was measured vertically and horizontally in mm using a caliper. According to [1] it is said that the diameter of the inhibition zone is in the range of 11-19 mm which is classified as having a strong inhibitory effect.

Table 1 Classification of Fungal Growth Inhibitory Responses

Clear zone diameter(mm)	Growth inhibition response
> 20 mm	Very strong
11 - 19 mm	Strong
5 - 10 mm	Currently
<4mm	Weak

Data Analysis Techniques, the research data that has been obtained were analyzed using the SPSS statistical application. The research data will be analyzed using Kruskal Wallis to test whether there is an effect of endophytic fungal isolates on the growth of Malessezia furfur. If there is an effect, it will be continued with Duncan's test to see the significant difference between each treatment.

#### **RESEARCH RESULT**

This research was conducted at the Pharmacy Microbiology Laboratory, BinaMandiri University, Gororontalo. Sampling of Chinese ketepeng (Cassia alata L) leaves was carried out in TanjungHarapan Village, Wonosari District, Boalemo Regency. This place was chosen because it has a geographical location in the mountains so that it produces plants that thrive and are abundant. Empirically, Chinese ketepeng leaves are widely used as a traditional treatment for tineaversicolor by the people in the village. Samples taken from the Chinese ketepeng plant are fresh leaves. Sampling was carried out in the morning.

Endophytic Fungus Isolation Results,Based on observations from 5 petri dishes that have been implanted with Chinese ketepeng (Cassia alata L) leaves, 1 petri dish is indicated to contain endophytic fungi. While the other dishes were not covered with endophytic fungi. One petri dish contained 2 isolates of endophytic fungi which were coded JEKA 1 and JEKA 2 because the two isolates had macro and microscopic differences as presented in (Table 2).

# **Table 2** Results of Endophytic FungusIsolation of Chinese KetepengLeaves (Cassia Alata L) .

No IsolateCharacteristics		Image
1. JEKA 1	Macroscopic: a. Dark gray color b. Smooth inner and outer structure c. Surface texture like cotton d. Spread colony edge	Endophytic

Microscopy :

a. Have spores that form like a chain
b. Have



No Zone Diameter Test Group Category		
Resista	ance (mn	1)
1. Control (+)	11	Strong
2. JEKA 1	10	Currently
3. JEKA 2	11	Strong
4. Control (-)	0	-

c. Magnification 100	)x
Macroscopic: 2. JEKA 2 a. White color b. Section structure inside and outside fine c. Texture surface like cotton d. Spread colony edg	Endoph ytic
Microscopy : a. Have spores b. Have hyphae growing elongate	spore

hyphae

c. Magnification 100 hvp

Note: JEKA = Chinese KetepengEndophytic Fungus

Potential Test Results of Endophytic Fungus Isolates from Leaves of Chinese Ketepeng Plant (Cassia alata L) on the Growth of Malassezia furfur. Based on the results of the potential test of the two solates against the fungus Malassezia Turfur, the average diameter of the inhibition zone was obtained as shown in Table 3

Table 3 Mean Inhibitory Zone Diameter(mm) Endophytic FungusIsolate Chinese ketepeng leafas antifungal

Note: JEKA	= KetepengEndophytic
Control $(+)$ = Ketoconazole	
Control (-) =Aquadest	

Based on Table 3, it shows that the average diameter of the inhibition zones in the positive control group and JEKA 2 group was greater than that of the JEKA 1 and negative control groups. Based on the results of the prerequisite tests that have been carried out, it can be stated that the parametric test cannot be carried out because the normality and homogeneity tests are not significant with the results of 0.091, 0.631, 0.041 not normality and 0.002 not homogeneous. Based on the decision making of the normality test and homogeneity test where if the significant value is 0.05 then the value is normal and homogeneous. but if the value 0.05 then the value is not normal and not homogeneous. Because the data in the prerequisite test is not normal, the method used in analyzing the statistical data uses the Kruskal Wallis non-parametric test. The results of this test produce a significance value of 0.05, which is 0.012,

After the analysis of hypothesis 1 test it was known that there was an effect of endophytic fungal isolates on the growth of Malassezia furfur, then further tests were carried out, namely the Duncan test. Duncan's test aims to analyze hypothesis 2, namely to determine the significant difference between each treatment of endophytic isolates in inhibiting or killing the growth of Malassezia furfur. Based on the Duncan test analysis, the results are shown in Table 4.

**Table 4** Results of Analysis Using<br/>Duncan's Test on Each<br/>Treatment.

No Test GroupAverage Symbol			
	(mm)		
1. Control (-)	0	а	
2. JEKA 1	8.33	b	

3. JEKA 2 9.17	b
4. Control (+) 10.67	b

Note: Unequal eye symbols (letters) show a significant difference

Based on Table 4.3, it can be seen that the treatment of negative control was significantly different from JEKA 1, JEKA 2 and positive control, while there was no significant difference between isolates of JEKA 1, JEKA 2, and positive control.

#### DISCUSSION

Endophytic fungi are fungi that grow and colonize plant tissues (hosts), especially in the roots, stems and leaves. Endophytic fungi can produce the same bioactive compounds and secondary metabolites as their host. This is thought to be because endophytic fungi undergo coevolution of genetic transfer from their hosts.

The ability of endophytic microbes to produce bioactive compounds is a very potential thing to be developed into herbal medicines. This is because endophytic microbes are microorganisms that are easy to grow, have a short life cycle and can produce large amounts of bioactive compounds by the fermentation method. another without causing any harm.

Based on the results of the isolation of endophytic fungi from the leaves of the Chinese Ketepeng plant (Cassia alata L), 2 of endophytic isolates fungi were obtained, each of which had a different colony morphology. [11] Suggested that endophytic fungi produced from host plant tissues can produce different types of isolates and in varying amounts, this is an adaptation mechanism of endophytes to the specific microbiology and physiological conditions of each plant so that it will affect differences endophytic colony composition and infection rate of

host plants occupied by endophytic fungi from the same location.

Based on macroscopic observations, it can be seen that the two isolates obtained have varying color and colony shape, namely JEKA 1 isolates are blackish gray, smooth inside and outside structure, have a surface texture like cotton, and have spreading colony edges, while in JEKA isolates 2 is white, the inner and outer structure is smooth, the surface texture is like cotton, and the edges of the colony are spread out.

Experts suggest that differences in the color of microbial colonies are influenced by intracellular pigments produced by these microbes, including carotenoid pigments, anthocyanins, melanin. tripyrylmethene and phenazim which will give different colors to each colony [18]. It has been shown that both endophytic fungal isolates have septate and nonseptate hyphae types. [22] Suggested that fungi that have septate hyphae type may to the class Phycomycetes belong (Zygomycetes and Oomycetes). Fungi that do not have septa are essentially scattered along the septa. The septate hyphae (coenocytic hypha)

The results of testing the antimicrobial activity of the fermented supernatant on the growth of Malassezia furfur showed that the two endophytic fungal isolates had an inhibitory effect on the test microbes, this was evidenced by the formation of a clear zone around the paper disk and strengthened by the results of the Kruskal Wallis statistical analysis test with Sig < value: 0.05 it can be concluded that H0 is rejected and H1 is accepted.

Based on the average value of the inhibition zone obtained from the measurement results on Malessezia furfur, it can be seen that the average inhibition zone for JEKA 1 isolates was 10 mm in the medium category, while JEKA 2 isolates was 11 mm in the strong category. This is in accordance with the statement

[19] which states that if the inhibition zone formed in the agar diffusion test is less than 5 mm in size, then the inhibitory activity is categorized as weak. If the inhibition zone measuring 5-10 mm is categorized as moderate, 10-19 mm is categorized as strong and 20 mm or more is categorized as very strong. Judging from the inhibition zone formed on the test microbe Malassezia furfur which is a partial inhibition zone.

Experts suggest that the inhibition zone is said to be total if the area around the disc is clear which indicates there is no longer growth of the test microbes, while the inhibition zone is said to be partial if the inhibition zone formed around the disc still contains several colonies of the test microbes [10].

In addition to using endophytic fungal isolates as a treatment on the test microbes, this study also used treatments in the form of negative control and positive control. In the negative control, there was no inhibition zone around the paper disk, this is because the negative control used was only distilled water, not an active compound that was able to inhibit the growth of Malassezia furfur. In contrast to the negative control, the positive control was used to inhibit the growth of Malassezia furfur using Ketoconazole. Based on the use of Ketoconazole as a positive control, an inhibition zone was seen around the paper disk which indicated that Malassezia furfur was inhibited.

Based on the results of further test analysis using Duncan's test, it showed that the negative control treatment had a significant difference with the positive control treatment, the treatment of JEKA 1 isolates, and JEKA 2. Meanwhile for the JEKA 1, JEKA 2, and positive control isolates there was no significant difference. Furthermore, based on the results of Duncan's further test that has been carried out, it is known that the inhibition zone for the inhibition of the growth of Malassezia furfur is found in JEKA 1 endophytic isolates with an average inhibition zone diameter of 10mm while for JEKA 2 endophytic isolates with an average inhibition zone diameter of 11mm.

The inhibition of the growth activity of Malassezia furfur was thought to be caused by the ability of endophytic fungal isolates to produce secondary metabolites. The secondary metabolites referred to are alkaloids, tannins, flavonoids, and anthraquinones. According to [6] alkaloid compounds work by inhibiting the biosynthesis of fungal nucleic acids, so that the fungus cannot grow and eventually die.

According to experts, the antimicrobial activity of tannin compounds by shrinking the cell membrane so that it interferes permeability with the of the cell membrane. As a result, cells cannot carry out living activities so that their growth is inhibited or even dies [2]. While phenolic compounds in the form of flavonoids and anthraquinones, can denature protein bonds in the microbial cell membrane so that the cell membrane becomes lysed, this will facilitate the entry of compounds into the cell nucleus [20].

Experts also state that the flavonoids in Chinese ketepeng have antimicrobial, anti-inflammatory, antioxidant, antiallergic effects and are effective for several groups of fungi or fungi [12]. The mechanism of flavonoids as antifungals is by disrupting mitochondrial hemostasis and also disrupting the permeability of cell membranes [4].

#### CONCLUSION

Based on the results of the study, it can be concluded that there is an effect of endophytic fungal isolates from the leaves of the Chinese Ketepeng (Cassia alata L) plant on the growth of Malessezia furfur. There was a significant difference between treatments, namely the negative control treatment was significantly different from JEKA 1, JEKA 2 and positive control, while the isolates of JEKA 1, JEKA 2, and positive control had no significant difference.

### REFERENCES

- Arbet A, Khotimah S, Yanti AH. 2014. Antibacterial Activity of Guava Leaf Extract (Dendropthoepentandra (L.) Miq) on the Growth of Salmonella typhi. Protobiont Journal. 3(2): 268-72. Tanjungpura University. Pontianak.
- [2] Ajizah, A. 2004. Sensitivity of Salmonella thypimurium to Psidiumguajava Leaf Extract L. Biology Education Study Program, pp. 31-38
- [3] Campbell, NA, JBR, lisa. AU, Michael
  I. C., Steven. AW, peter. VM Robert.
  BJ 2008. Wulandari Translation, d. T.
  Biology Eighth Edition Volume 2.
  Erlangga Publisher. Jakarta
- [4] Freiesleben,SH and Jager,AK Correlation Between Plant Secondary Metabolites And Their Antifungal Mechanisms. A Review, Med Aromat Plants 3: 154, 2014.
- Hasanah, U., Rimayati, [5] and Idramsyah. 2015. Antifungal test Pathogens of fungal secondary metabolites extract. Journal of Bioscience, 6-12.
- [6] Julianto. Khotimah, S., Raharjo, W. 2015. Antifungal Activity Test of Ethanol Extract of Langsat Fruit Seed (Lansiumdomesticum corr.) Against Candida albicans In Vitro. Pontianak. Tanjungpura University, Pontianak.
- [7] Be strong, Arifah. 2013. Inhibitory Test of Chinese Ketepeng Leaf Extract (Cassia alata L) Against the Growth of Pseudomonas aeruginosa and Streptococcus muntas Bacteria. Purwokerto ; Faculty of Teacher Training and Education, University of MuhamadiyahPurwokerto.

- [8] Leman, A,. Michael. 2017. Inhibitory Test of Papaya Leaf Extract (Carica papaya) Against Candida albicans.
- [9] Maheasy, R., Abdullah, N., Pratiwi, A., Hidayah, M., Ismail, R., 2018. Test the Effectiveness of Ethanol Extract on Dau.
- [10] Muhammad, ZK 2014. Antibacterial Activity Test of Sintok Leaf Extract and Fraction (CinnamomumsintocBlume.) against Staphylococcus aureus and Pseudomonas aeruginosa and Analysis of Components of Active Fraction Compounds by Gas Chromatography-Mass Spectrometry. (Essay). Pharmacy Study Program. Faculty of Medicine and Health Sciences. Jakarta
- [11] Noverita, D. Fitria, and E. Sinaga. 2009. Isolation and Antibacterial Activity Test of Endophytic Fungus from Leaves and Rhizomes of ZingiberOttensii Val. Indonesian Pharmacy Journal Vol. 4 No. 4th July 2009: 171-176
- [12] Daughter, DelviRusitaini. 2016. Comparison of the Effectiveness of Terbinafine With Chinese Ketepeng Leaf Extract (Cassia alata L) Against Fungal (Malassezia furfur) Growth as Etiology of Pityriasisversicolor. Faculty of Medicine, University of Lampung: Bandar Lampung.
- [13] Priharta A. 2008. Isolation and Identification of Endophytic Bacteria in the Stem of Artemisia annua l. Tested for its Antibacterial Potential Against Eschericia coli and Staphylococcus aureus. Essay. Faculty of Pharmacy. Sanata Dharma University. Yogyakarta.
- [14] Pratiwi, ST 2008. Pharmaceutical Microbiology. Erlangga. Jakarta
- [15] Rajai, L, Ahmad, I, Hasiani, V. 2015. Isolation of Endophytic Fungus and Production of Antioxidant Secondary Metabolites from Girlfriend Leaves

(LawsoniaInermis L). Mulawarman University. East Kalimantan.

- [16] Suarni, Panggeso, J, Rosmini. 2017.
  Inhibitory Test of Chinese Ketepeng Leaf Extract (Cassia alata L.) Against Pathogenic Fungus Phytophthorapalmivora that Causes Cocoa Fruit Rot Disease (Theobromae cacao L.). Hammer ;Tadulako University.
- [17] Simarmata, R, S. Lekatompessy, and Sukimah, H. 2007. Isolation of Endophytic Microbes from Medicinal Plants Connecting Life (Gymuraprocumbens) and Analysis of Potential as Antimicrobials. BerkPenelHayati. Vol. 3 (13): 85-90.
- [18] Safrida, Yuni. 2012. Isolation and Characteristics of Potentially Probiotic Bacteria in Mackerel (Rastrelligersp) Journal. Syiah Kuala University: Aceh.
- [19] Sirait, B, R, G, Susilorini, E, T, Surjowardojo, P. 2015. Inhibitory Power of Manalagi Apple Peel (Malussylvesters mill.) Against the Growth of Staphylococcus aureus and Pseudomonas sp. Causes of Mastitis in Dairy Cows. Poor. Brawijaya University.
- [20] Sulistyawati, D., and S. Mulyati.
   2009.Test activity of cashew leaf infusion (Anacardiumoccidentale L.) against Candida albicans.
   Biomedicine.; 2(1): 47-51.
- [21] Tan, R., X., Dan Zou, W., X. 2001. Endophytes : a rich source of functional metabolites. Natural product reports, 18(1993), pp. 448-459.
- [22] Waluyo, L. 2007. General Microbiology. University of Muhammadiyah Malang

Jurnal Ilmiah Dr. Aloei Saboe (JIAS) 4059

[23] Zulkoni, S. 2011. Parasitology, Yogyakarta: NuhaMedika.