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Effectiveness of Ethanol Extract among Three Ganoderma Species From Lombok in Inhibition Growth of *Candida albicans* and *Cryptoccocus neoformans*

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Abstract: *Candida albicans* and *Cryptoccocus neoformans* are opportunistic pathogenic fungi that cause infectious diseases that are the world's biggest health problems. The use of antibiotics is one way to overcome the spread of the infection and cause microbial resistance. Ganoderma is one of the many macrophages found on Lombok's island, and studies of its antifungal activity have not been carried out. The purpose of this study was to determine the antifungal potential and the effect of different concentrations of ethanolic extracts of three Ganoderma species on *C. albicans* and *C. neoformans*. Ganoderma samples were obtained from Suranadi Taman Wisata Alam (TWA), Sesaot TWA, Tunak Mountain TWA, Kerandangan TWA, and Pusuk Forest. Ganoderma extraction was carried out by the maceration method using ethanol 95% solvent. The extract concentrations used are 20%, 40%, 60% and 80%. This research was conducted using the wells method with metronidazole as a positive control and 50% DMSO as a negative control. The parameter measured is the large diameter of the inhibition zone formed around the well. The results obtained are the three species of Ganoderma have antifungal activity against test fungi, and different levels of concentration affect inhibition. The amount of the inhibition zone is directly proportional to the high concentration of the extract. All three Ganoderma species are more effective in inhibiting the growth of *C. neoformans* compared with *Candida albicans*.

Keywords: maceration; resistance; Taman Wisata Alam; accompanying infections

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Introduction

The fungal disease has claimed more than 1.5 million lives and affected more than one billion people. However, yeast infection is still a topic neglected by public health authorities even though most deaths are avoidable from fungal diseases. Fungal severe infections occur due to other health problems, including asthma, AIDS, cancer, organ transplants, and corticosteroid therapy [1]; [2].

Recently, 3,000,000 cases of chronic pulmonary aspergillosis were found, and 223,100 cases of cryptococcal meningitis accompanying infections in people with HIV/AIDS. There are around 700,000 cases of invasive candidiasis, around 500,000 cases of pneumocystis jirovecii pneumonia. There are around 250,000 invasive aspergillosis cases in people with HIV/AIDS. Each year, there are approximately 700,000 cases of invasive candidiasis, 500,000 cases of pneumocystis jirovecii pneumonia, 250,000 cases of invasive aspergillosis, 100,000 cases of spread of

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Histoplasma, more than 10,000,000 cases of fungal asthma, and approximately 1,000,000 cases of fungal keratitis [3], [4], [5].

Fungal diseases are also the most common comorbid infections in people with AIDS, especially candidosis caused by Candida sp. The cryptococcal disease is caused by Cryptoccocus neoformans, aspergillosis by Aspergillus sp. The histoplasmosis by a fungal pathogen, namely Histoplasma capsulatum [6]. The first three fungi are opportunistic. Parasitic and fungal infections affecting people with AIDS will increase morbidity and mortality.

Candida albicans is a pathogenic fungus that is very dangerous for immunosuppressive patients because of the fungus's opurtunistic nature. Also, Candida species form colonies in the vagina of at least 20% of all women. Statistical tests show an increase in Candida species, especially *Candida albicans,* up to 30% at the end of pregnancy and immunosuppressed patients. Factors that cause Candida attacks are decreased body defense, gene polymorphism, allergic factors from the host, serum glucose levels, antibiotics, psychosocial stress, and estrogen that affect Candida vulvovaginitis's risk. *Candida albicans* are capable of forming blastospores, pseudomycelia, actual mycelia, and chlamydospore [7].

The use of antibiotics is one way to overcome the spread of the infection. Antibiotics as drugs to treat infectious diseases, their use must be rational, appropriate, and safe. Irrational use of antibiotics will have a negative impact, such as the occurrence of immunity of microorganisms against some antibiotics, increased side effects of drugs [8].

Research on a compound's activity as an antifungal is an initial step to provide important information as an effort to overcome a disease resistance caused by fungi. Several types of fungi, especially from the Ganoderma genera, have long been known as a source of medicine because of their bioactive compounds [9], [10].

Studies on the bioactive content and potential of Ganoderma that inhabit Lombok island's forest area as a source of medicine have not been done much. Even though Lombok Island's uniqueness in the transitional zone between West Wallace and East Wallace is thought to affect the composition and bioactive composition of Ganoderma. This is possible because the Ganoderma host plant's character in the transition zone represents the properties found in the two clamp zones.

This paper presents the results of research on the effectiveness of ethanol extracts from *Ganoderma Lucidum, Ganoderma applanatum*, and *Ganoderma* sp. originating from the Suranadi Nature Tourism Park (TWA), Sesaot TWA, Tunak Mountain TWA, Kerandangan TWA, and Pusuk Forest in inhibiting the growth of *Candida albicans* and *Cryptoccocus neoformans*.

Method

Sample preparation.

Samples of Ganoderma Lucidum, Ganoderma Applanatum, and Ganoderma sp., was taken from the Suranadi Taman Wisata Alam (TWA) forest area, Sesaot TWA, TWA, Tunak Mountain TWA, Kerandangan TWA, and Pusuk Forest. Intake was done by cutting the Ganoderma mushroom fruit's body and then putting it in a zip lock or sterile plastic [11]. Furthermore, the sample is cleaned of all the dirt attached, dried, and cut into small pieces, and then roasted at a temperature of 40°C to dry.

Sample extraction

Samples were blended and macerated using 95% ethanol solvent for five days in vial tubes, which were tightly closed with regular stirring and carried out every day [12]. After that, it was filtered, and the solvent is evaporated using an evaporator. After that, the results of maceration are filtered to obtain maserate. The ethanol maserate obtained was then concentrated using an evaporator at 40°C. The viscous extract was dissolved using 50% DMSO according to the concentration for testing, namely 20%, 40%, 60%, and 80% [13].

Percentage of Yield Extract

The yield is the percentage of raw material parts that can be used or utilized with the total raw materials, so the calculation of the percentage of yields is as follows:

$$\%\text{Rendemen} = \frac{\textit{Berat Ekstrak Kental (gr)}}{\textit{Berat Simplisia (gr)}} \times 100\%$$

Making Sabouraud Dextrose Agar (SDA) Media

In this study, the media was Sabouraud Dextrose Agar (SDA) with a composition of 27.5 gr Dextrose, 12.5 gr of Nutrient Agar, and 5 gr of Peptone mixed with 500 ml of distilled water in Erlenmeyer then heated while stirring until boiling on a hot plate. After boiling, the media is sterilized by autoclaving at 121°C and 2 atm pressure for 30 minutes.

Preparation of Fungal Test

Rejuvenation of the test fungi (*Candida albicans* and *Cryptococcus neoformans*) was carried out by means of existing pure isolates, taken one ose and then etched on the SDA medium in a petri dish using the quadrant scratch method and incubated at 30°C for 24 hours

Bioactivity Test of Ganoderma Ethanol Extract

Antifungal bioactivity testing was carried out using the diffusion method, so that is the wells method. This method is used to ensure the presence of antifungal activity found in *Ganoderma* sp. The parameter used is the inhibitory zone.

The extract solution was made with several concentrations of 20%, 40%, 60% and 80% v/v using 50% DMSO solvent. Suspension of the test fungus that has been adjusted to the turbidity of Mc Farland was etched using a cotton swab over the SDA media until it is even (covering the entire surface of the test media), then a hole was made in the media with a diameter of 7 mm using a sterile hole. Each well was piped as much as 100 µL extract from each concentration that was made. After that, it was incubated at 30^{o}C. Repetition is done three times. The positive control used in the form of antibiotics is 200 mg of metronidazole synthesis.

Result and Discussion

Extract of Three Ganoderma Species

Sample preparation of three Ganoderma species was obtained by dry sample (simplicia) in the form of powder to expand the sample's surface in contact with the solvent so that the results obtained were greater in extraction. The following table of simplicia and the percentage of marinade obtained from the extraction results (Table 1).

Table 1. Percentage of Yield of Ethanol Extract from Three Ganoderma Species.

Ganoderma species	Simplicia (gr)	Extract (gr)	The yield of extract (%)
Ganoderma applanatum	192,8	6	3,11
Ganoderma lucidum	209,6	5	2,39
Ganoderma sp.	182,3	4	2,19

The above table shows that the extraction results obtained are very few. So, as to get even more extracts needed quite a lot of samples too. The *Ganoderma applanatum,* extraction results were obtained in a very thick solution with a blackish brown color. The *Ganoderma lucidum* in the form of a viscous brown solution in dark brown and *Ganoderma* sp. in the form of a thick yellowish-brown solution, and there are lumps of dark brown.

Antifungal Activity of Ethanol Extract of Three Ganoderma Species

Based on the test results, there are inhibitory zones in both fungi (Figure 1), which means that the extract of *Ganoderma lucidum*, *Ganoderma applanatum,* and *Ganoderma* sp. can inhibit the growth of *Candida albicans* and *Cryptoccocus neoformans*.

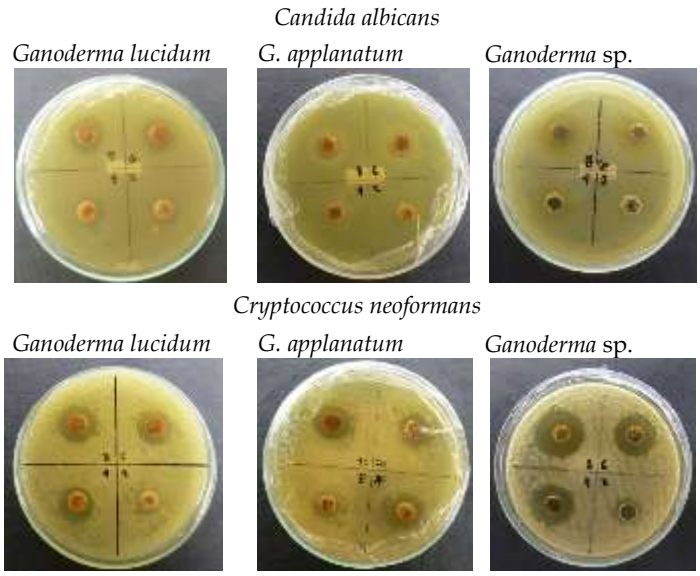

Figure 1. Inhibition zone results of ethanol extract testing from three Ganoderma species.

The following is a graph of the inhibitory zone measurement results for the antifungal activity of ethanol extracts of the three Ganoderma species against test fungi:

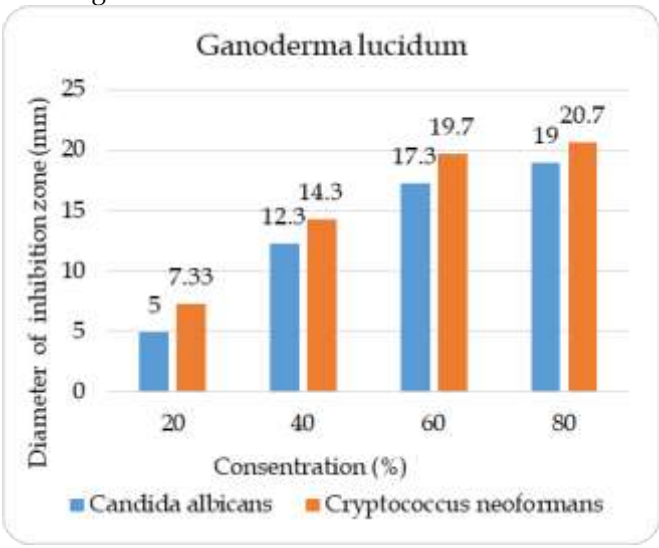

Figure 2. Test results of the antifungal activity of ethanolic extract of *Ganoderma lucidum* against *Candida albicans* and *Cryptococcus neoformans*, repetition was performed three times

Figure 2 shows that the ethanol extract of *Ganoderma lucidum* has antifungal activity against *Candida albicans* and *Cryptococcus neoformans*. However, the antifungal activity of this extract is more effective against *Cryptococcus neoformans*.

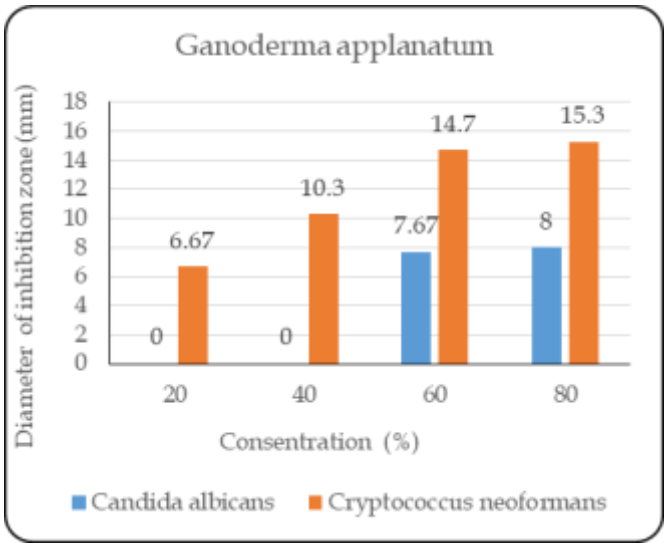

Figure 3. Test results of the antifungal activity of ethanolic extract of *Ganoderma applanatum* against *Candida albicans* and *Cryptococcus neoformans*, repetition was performed 3 times

Figure 3 shows that the ethanolic extract of Ganoderma applanatum has antifungal activity against *Candida albicans* and *Cryptococcus neoformans*. The antifungal activity of this extract is more effective against *Cryptococcus neoformans*, it is clear that *Candida albicans* activity is very low, even at concentrations of 20% and 40%, which do not form inhibition zones.

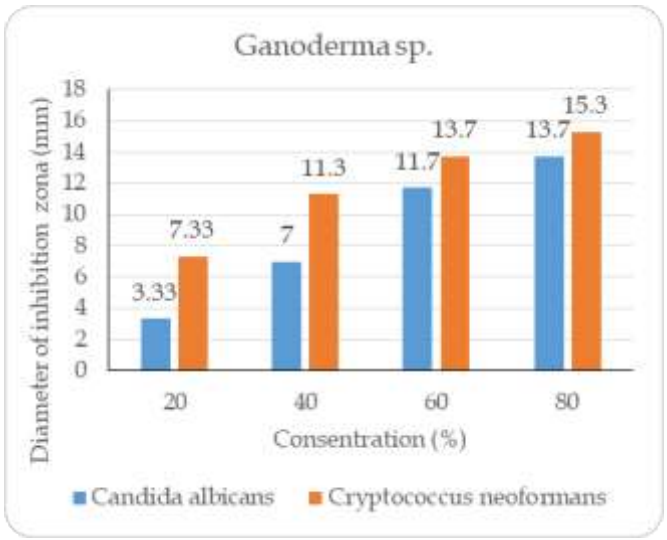

Figure 4. Test results of the antifungal activity of ethanolic extract of *Ganoderma* sp. against *Candida albicans* and *Cryptococcus neoformans*, repetition was performed three times

Similar to the two previous species, the extract of *Ganoderma* sp. gave positive results, marked by the inhibitory growth of *Candida albicans* and *Cryptococcus neoformans*. This extract is also more effective in inhibiting the growth of *Cryptococcus neoformans*.

The graph below illustrates ethanol extracts of the three Ganoderma species to inhibit the growth of *Candida albicans* and *Cryptococcus neoformans*.

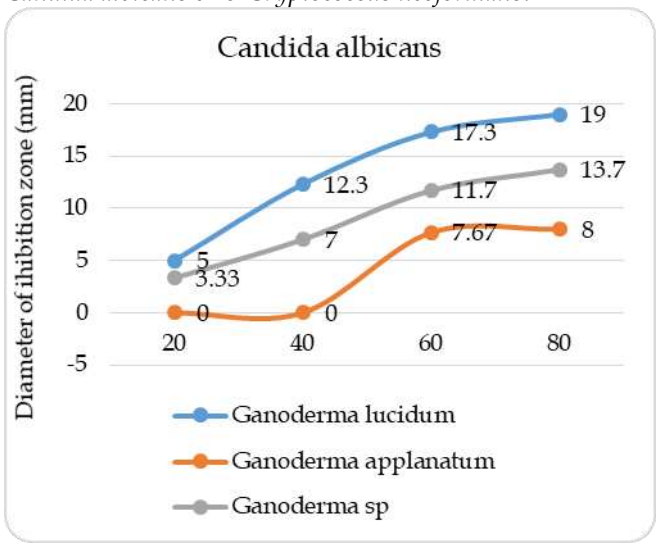

Figure 5. Test results of the antifungal activity of three ethanolic extracts of three Ganoderma species against *Candida albicans*

Figure 4 above shows the considerable difference between the three Ganoderma species in their inhibition of *Candida albicans*. *Ganoderma applanatum* has the lowest inhibition compared to the two other species and the highest inhibition against *Candida albicans* is *Ganoderma lucidum*.

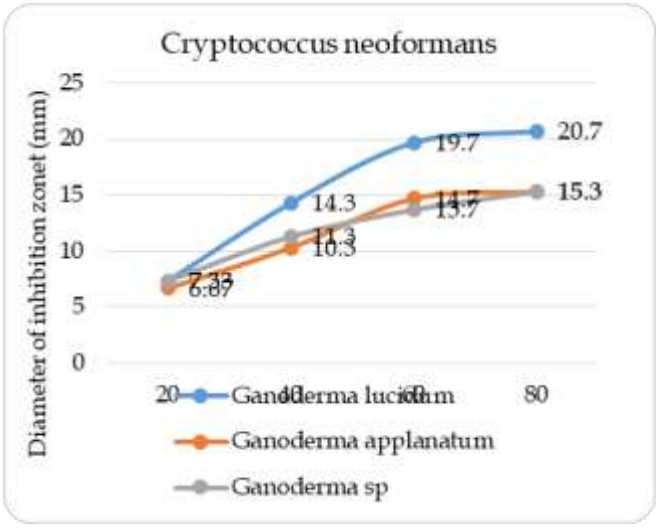

Figure 6. Test results of the antifungal activity of three ethanolic extracts of three Ganoderma species against *Cryptococcus neoformans*

Figure 6 shows the inhibitory properties of the three Ganoderma species. The difference between the three is not too far, like *Candida albicans*. *Ganoderma applanatum* has the lowest inhibition, and *Ganoderma lucidum* has the highest inhibition against *Cryptococcus neoformans*.

Figures 2, 3 and 4 show that the higher the concentration level of the extract, the greater the inhibitory zone formed, which means that the high concentration is directly proportional to the inhibition zone's magnitude. It happens because the active compound contained more than the active compound contained in a solution whose concentration is lower at high concentrations. Therefore, the higher concentration of inhibition is greater, and the concentration level of the extract with the greatest inhibition is the highest concentration of 80%. The results of Swati [14] also showed that the higher the concentration of *Ganoderma lucidum* extract, the higher inhibition of *Candida albicans* growth. It is consistent with the opinion of Triastinurmiatiningsih [15], which states that the size of the inhibitory zone is influenced by the level of sensitivity of the test organism, culture media, and incubation conditions, the diffusion rate of the antifungal compound and the concentration of the antifungal compound.

The graph above also shows that the antifungal inhibition test by 96% ethanol extract can inhibit test fungi, namely *Candida albicans* and *Cryptococcus neoformans*, in which all three extracts are more effective against *C. neoformans* than *C. albicans*. This occurs because C. albicans is a normal microbiota that is often exposed to chemicals (such as antibiotics), so it mutates and has a physiological effect. For example, eliminating compounds or neutralizing antifungal properties, so that they become resistant to some antifungal compounds or have a morphological impact, such as forming protective capsules in the form of biofilms. According to Brand [16], Biofilm fungi play a role in fungi' defense and virulence. Biofilm is a fungal colony that forms an organic polymer matrix consisting of two layers, namely a thin basal layer, which is a layer of the yeast itself, and an outer layer of hypha layer, which is thicker but the structure is more tenuous.

Graphs 5 and 6 show that ethanol extract from *Ganoderma lucidum* has greater antifungal activity against *Candida albicans* and *Cryptococcus neoformans* than *Ganoderma applanatum* and *Ganoderma* sp. It is presumably because the active compound as an antifungal in *G. Lucidum* has a higher amount than the other two Ganoderma fungi. The results of this study are in line with the results of research by Kumar [17], which shows that *G. lucidum* has antifungal activity against *Candida albicans, Aspergillus flavus, A. Fumigates, and Crytococcus neoformans*. This fungus has also been used long enough as a medicine for various diseases. This study justifies the claimed uses of *G. lucidum* in the traditional system of medicine and its bioactive components to treat various infectious diseases caused by the microbes.

From all the above results, it is known that all three Ganoderma species contain antifungal compounds, where the antifungal compounds contained in Ganoderma include terpenoids, flavonoids, tannins, alkaloids, and steroids [18], [19], [17]. These antifungal compounds work like antibiotics.

The mechanism of flavonoids' action in inhibiting fungal growth is by disrupting fungal cell membrane permeability. Hydroxyl groups found in flavanoid compounds cause changes in organic components and nutrient transport, leading to toxic effects on the fungus [20].

Alkaloids can inhibit nucleic acid synthesis and affect ergosterol in fungi. In contrast, terpenoids can dissolve lipids in fungal cell membranes and interfere with nutrient transport, causing cell membranes to be malnourished, resulting in damage to fungal cells [21].

Conclusion

Ethanol extract of *Ganoderma lucidum, Ganoderma applanatum,* and *Ganoderma* sp. has an antifungal activity that inhibits Candida albicans' growth *Cryptococcus neoformans.* Ethanolic extract of *G. lucidum* has higher fungal inhibitory activity than G. applanatum and Ganoderma sp. The three Ganoderma ethanol extracts were more effective in inhibiting *C. neoformans* than *C. albicans*. Extract concentration is directly proportional to the amount of inhibition zone formed. This study justifies Ganoderma's claimed uses in the traditional system of medicine and its bioactive components to treat various infectious diseases caused by the microbes.

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