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Antibacterial Activities of Ethanol Extracts Fruit Bodies of *Ganoderma lucidum* and *Rigidoporus microphorus* Against *Escherichia coli* and *Staphlyococcus aureus*

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Abstract

Ganoderma lucidum and *Rigidoporus microporus* are two examples of macro fungi that are commonly found in the highland rain forest of Indonesia, and are even found in lowlands such as at urban forests in DKI Jakarta. Many of these macro fungus have been reported as potential medicinal substances, especially from the species of*G. lucidum*, whose fruit bodies are usually obtained from forests in upland areas. The aims of this study were to determine the antibacterial activity of the fungi *G. lucidum* and *R. microporus*from several places in South Jakarta against the growth of *E. coli* and *S. aureus* bacterias. Testing for antibacterial activity was carried out using the Kirby-Bauer diffusion method. The results showed that the extract of *G. lucidum* fruit bodies were only able to inhibit the growth of *S. aureus* bacteria. While the fruit body extract of R. microporus was able to inhibit both types of tested bacterias, i.e*E. coli* and *S. aureus*. The results showed that *G. lucidum* extract were only able to inhibit of *S. aureus* bacteria. Meanwhile *R. microporus* extract was able to inhibit both *E.coli* and *S.aureus.* There was no significant effect of increasing the concentration of mushroom fruit extract on the inhibition zone of the tested of bacteria.

Keywords: Antibacterial, diffusion, *G. lucidum*, inhibition zone, *R. Microporus*.

Introduction

Ganoderma lucidum and *Rigidoporus microporus* are two examples of macro fungus that have relatively large in body sizes. These fungus are commonly found in Indonesian forests both in the highlands(Noverita *et al*., 2017, Noverita *et al.*, 2019), and in the lowlands ((Noverita et al., 2018, Wati *et al*., 2019). Macro fungus have a fruiting body structure that consisted of a hood, blade / pore, with or without stalks, rings, and volva(Darwis *et al*., 2011).Environmentally, the growth of fungi is strongly influenced by several factors, such as temperature, humidity, and light intensity(Tampubolon, 2010).

G. lucidum is known to the public as Lingzhi mushroom. *G. lucidum* belongs to the Ganodermataceae family and the Basidiomycetes class. This saprophytic fungui grows / attaches itself to dead or rotting trees, such as oak, maple, elm, willow and magnolia(Orole and Biotechnology, 2016).*G. lucidum*mushroom has been widely used as a medicinal to treat various diseases since thousands of years ago, including cancer, tumors, hypertension, microbial infections, and inflammation. Besides that, many studies have been conducted and developed for the discovery of new drugs and testing the pharmacological effects of *G. lucidum* for human health(Paterson, 2006).Furthermore,Styafitri *et a*l. (2006) have conducted research on the antibacterial activity of *G. lucidum* distilled water extract against the *Proteus* sp. using the diffusion method, and as a results indicated that the distilled water extract of *G.*

lucidum was able to inhibit the growth of *Proteus* sp. bacteria. with an average diameter of the inhibition zone 8.03 mm; 8.2 mm; 9.4 mm; 9.8 mm; and 10.3 mm, respectively.

R. *microporus* is a fungus that belongs to the Basidiomycetes class and the Polyporaceae family(Alexopoulos et al., 1996). This fungus is also known as the White Root Mushroom which causes white root disease in several plants such as cashews, cloves, and rubber(Shofiana et al., 2015, Rahayu *et al.*, 2017).The antibacterial activity of ethyl acetate extracts of several types of basidiomycota including *Rigidoporus lineatus* (URM 6828) and *R. microporus* (URM 6878) obtained from Northeast Brazil against eleven strains of *Staphylococcus aureus*, showed that those fungi were able to inhibit the growth of eleven strains of *S. aureus* with the zone of inhibition ranged from 9 to 24 mm and the Concentration Minimum Inhibition rate (MIC) was between 0.18–147 μg / mL(Ferreira-Silva *et al.*, 2017).

Judging from the existing potential of the two species of macro fungi (*G.lucidum* and *R. microporus*) as antibacterial agents, and their fairly wide growth distribution, it is suspected that there is a difference in the activity of these fungi based on differences in the places where they grow. Therefore, this research was carried out with the aim of knowing the antibacterial activity of the macro fungi *G.lucidum* and *R. Microporus* obtained from the Camping Ground Park and campus yard of Universitas Nasional Pejaten, Pasar Minggu Campus, in South Jakarta on the growth of *E. coli* and *S. aureus*. The antibacterial activity test was carried out using the Kirby-Bauer diffusion method with different concentrations.

The hypothesis proposed in this study are:

1. The ethanol extract of *G. lucidum* and *R. microporus* was able to inhibit the growth of the tested bacteria.
2. There are differences in the ability to inhibit *G.lucidum* extract and *R. Microporus* extract against bacteria
3. There was an increase in the inhibition zone in line with the increase in the concentration of the mushroom extract used.

Materials And Methods

Time and Place of Research

This research was conducted from May - August 2019. The research samples were taken at the Ragunan Campground and the campus yard of Universitas Nasional Pejaten, Pasar Minggu, South Jakarta. Fruit body extraction and antibacterial activity tests were carried out at the Laboratory of Microbiology and Genetics, Universitas Nasional , Jl. Bambu Kuning, Pasar Minggu, South Jakarta.

Tools and Materials

The tools used in this research includes: aluminum foil, autoclave, beaker glass, blender, bulb, petri dish, funnel, measuring cup, scissors, calipers, filter cloth, electric stove, erlenmeyer flask, laminar air flow, light microscope, balance scales, knives, measuring pipettes, test tube racks, rotary evaporators, test tubes, and analytical scales.

Materials that will be used in this research includes: the fruit bodies of macro fungi *G. lucidum* and *R. microporus, E. coli* and *S. aureus,* a collection of the Laboratory of Microbiology and Genetics Universitas Nasional. Nutrient Broth (NB) and Mueller Hinton Agar (MHA) media, aqueous solution, 96% and 70% ethanol, McFarland, 0.85% NaCl, chloramphenicol discs, cotton, disc paper, wrapping paper, label paper, rubbing alcohol, markers, and cotton swabs.

Sample Preparation

The fruit bodies of the macro fungi *G. lucidum* and *R. microporus* that grow on the Ragunan Campground and the campus yard of Universitas Nasional Pejaten are observed for their fruit bodies and photographed to be identified, then taken and cleaned, then cut into small pieces, dried and blended until smooth.

Sample Extraction

The extraction process is carried out by the maceration method with a ratio of 1: 4 between the sample and the solvent(Handayani and Nurcahyanti, 2014). A total of 100 g of sample was immersed using ethanol (96%) solvent in an Erlenmeyer flask. The sample was macerated for 2 x 24 hours, then the extract solution was filtered and the residue was soaked again with 96% ethanol solvent for 2 x 24 hours. The extract solution was evaporated using a rotary evaporator at 40ºC.

Preparation of extract concentration

The concentrations used in this study were 900, 600, and 300 mg / mL as has been modified (Zahro and Agustini, 2013). The extract was weighed as much as 0.9 g, 0.6 g, and 0.3 g then each extract was dissolved in 96% ethanol until the volume was 1 mL.

Antibacterial Activity

Antibacterial activity testing was carried out by the paper disc diffusion method. In this method, filter paper discs (paper disks) that have been dripped with the fruit body extract of the *G. lucidum* and *R.microhorus* fungi are used according to the concentration that has been made. The disc paper is then placed in a Petri dish containing the media that has been inoculated with the test microbes, then incubated in an incubator for 18-24 hours at 37ºC. The observations obtained were indicated by the presence or absence of a clear area formed around the disc paper indicating the zone of inhibition against bacterial growth measured using a caliper.

Data Analysis

This study used a completely randomized design (CRD) with 2 treatments, namely: mushroom extract (T1) and test concentration (T2). The research was conducted in 3 repetitions. The calculations were performed using SPSS 22.

Results and Discussion

Description of *Ganoderma lucidum*

The fruit of *G. lucidum* grows at the base of a livetree as a parasite. The pileus hood is half a circular, like a kidney, convex to concave, short-stemmed, thick, cork-like texture and hard, size of 10-25 cm, 3-5 cm thick, 3-8 cm long of stem. The surface color of the fruit is light brown to dark brown with a white border. The bottom of the hood is porous, tightly whitish to light brown (Figure 1). Chang and Miles (1989)stated that *G. lucidum* has a fruiting body that is thick, foamy and reddish yellow at first and then turns brownish in color as it ages. The border of the fruiting body is usually thin white at the beginning and becomes light brown in the later stages. The shape varies round, semi-circular and fan-like or kidney-like,further according to Wang et al. (2012), the fungus *G. lucidum* has a pileus shaped like a kidney to half a circle, convex to concave, the stalk is very short, the attachment of the stipe to the pileus varies from lateral to almost to the center, the surface of the pileus with radial

grooves or with concentric zones, the thickness of the pileus appears. from one layer to several layers; the color of the pore surface varies from whitish to yellowish.

Figure 1. Fruit bodies of Ganoderma lucidum

The fruiting body of *R. microporus* is fan-shaped, rather thick, slightly hard and slightly woody, the surface of the hood with growth zones, the edges of the fruit body are thin, and not stemmed. The surface color of the fruit body is orange yellowish to brown. The lower surface is found with pores where spores are produced, orange-brown in color, smooth (Figure 2).According to Semangun (2008);Kaewchai *et al*. (2010), fruit bodies are thick fan-shaped, slightly woody, have growth zones, thin edges, not stemmed, orange to brownish red with slightly prominent dark zones, smooth and orange-brown under surface, fine pores.

Figure 2. Fruit bodies *Rigididoporus microporus*

Antibacterial Activity of the Ethanol Extract of *G. lucidum* and *R. microporus*

The antibacterial activity of the ethanol extract of the macro fungi *G. lucidum* and *R. microporus* against *E. coli* which represents Gram negative bacteria, and *S. aureus* which represents Gram positive bacteria is shown in Table 1.

Tabel 1.Inhibition zone diameter of *G. lucidum* and *R. microporus* fruit body extracts against *E. coli* and *S. aureus* test bacteria

Sample	Concentratios test (mg/mL)	Zone of Inhibition (mm)							
		E.coli			Average	***S.aureus***			Average
		1	2	3		1	2	3	
Ganoderma lucidum	900	6,00	6,00	6,00	6,00	6,8	6,5	6,6	6,63
	600	6,00	6,00	6,00	6,00	6,2	6,5	6,6	6,47
	300	6,00	6,00	6,00	6,00	6,00	6,00	6,00	6,00
Rigidoporus microporus	900	8	7,5	7,3	7,60	6,6	6,2	6,5	6,47
	600	6,4	6,4	6,5	6,43	6,00	6,00	6,00	6,00
	300	6,2	6,2	6,3	6,23	6,00	6,00	6,00	6,00
Positive control	-	25,45	26,40	26,35	26,07	19,80	19,80	19,80	19,80
Negative control	-	6,00	6,00	6,00	6,00	6,00	6,00	6,00	6,00

Note: Size 6.00 mm indicates no growth inhibition zone.

Table 1shows that the fruit body extract of *G. lucidum*was only able to inhibit the growth of *S. aureus* bacteria at concentrations of 900 and 600 mg / mL with an average inhibition zone diameter of 6.63 and 6.47 mm (Figure 3). Meanwhile, the fruit body extract of *R. microporus* was able to inhibit the growth of the two tested of bacteria (e.g., *E.coli* and *S. aureus*), with mean diameter of the inhibition zone against *E. coli* bacteria, from concentrations of 900, 600, and 300 mg / mL were 7.60, 6.43, and 6.23 mm, respectively. Meanwhile, for *S. aureus* bacteria only at a concentration of 900 mg / mL, with an average diameter of the inhibition zone of 6.47 mm (Figures 4 and5).

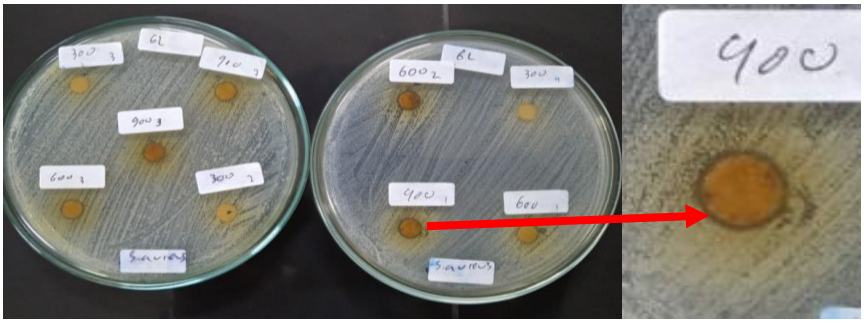

Figure 3. Inhibition Zone of fruit body extract of *G. lucidum* against *S.aureus*

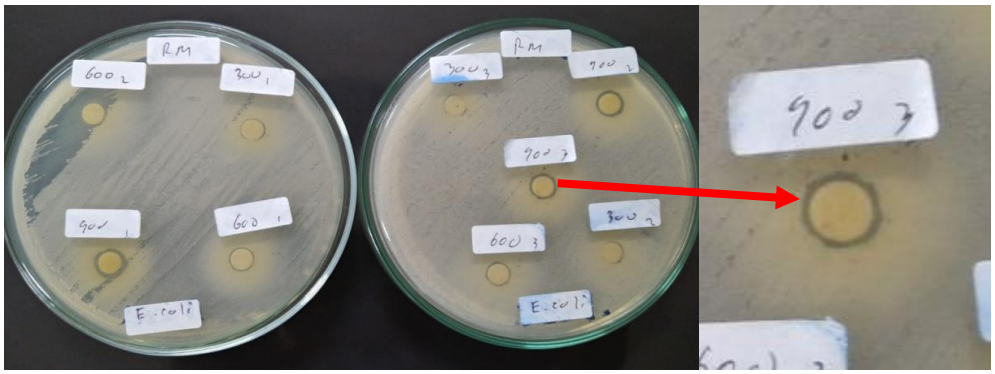

Figure 4. Inhibition Zone of fruit body extractof *R.migroporus*against *E.coli*

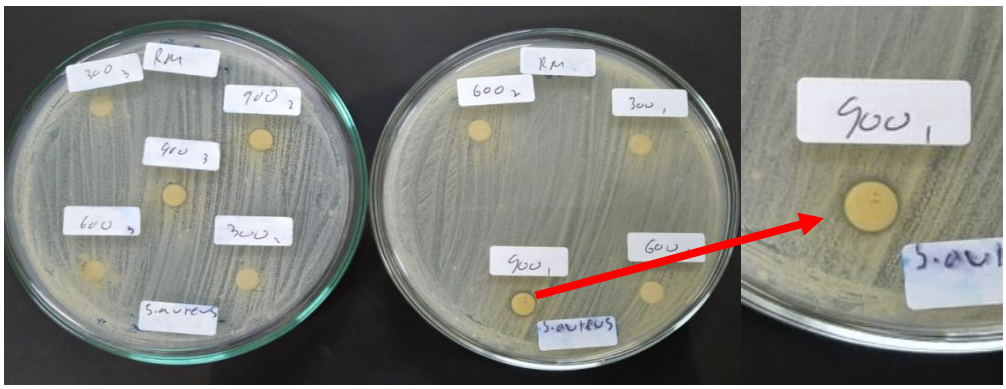

Figure 5. Inhibition Zone of fruit body extractof *R.migroporus*against *S.aureus*

The results of the analysis showed that the fruit body extracts of *G. lucidum* fungihada significance correlation(Tukey test, $p < 0.05$) only for *S. aureus* bacteria. This means that the extract of *G. lucidum*mushroom body has ability to inhibit the growth of *S. aureus* bacteria. Likewise, *R. microporus* mushroom body extract had a significance correlation(Tukey test, $p < 0.05$) for both types of tested bacteria (*E. coli* and *S. aureus*), which means that the body extract of *R. microporus* mushrooms was able to inhibit growth. *E. coli* and *S. aureus*.

The capable to inhibit the fruit body extracts of *G. lucidum* and *R. microporus* against the tested of bacteria used is related to the content of secondary metabolites produced by each of these mushroom fruit bodies. According to Handrianto and Science (2018), *G. lucidum* mushrooms containsof active compounds, such as triterpenoids, alkaloids, steroids, and coumarin which are antibacterial.Furthermore, according to Falade *et al*. (2017), *R. microporus* mushrooms contain of compounds, such as tannins, saponins, terpenoids, flavonoids, steroids, and cardiac glycosides.Those compounds are antibacterial(Doss *et al*., 2009, Sukadana, 2010, Mariajancyrani *et al*., 2013, Akinpelu *et al*., 2014).

When we compared to the ability of the two species of mushroom fruit body extracts in inhibiting the growth of the tested bacteria, it appears that the fruit body extract of *R. microporus* is more effective, because it can inhibit the growth of the two types of tested bacteria (*E. coli* and *S. aureus*).Despite of the ability to inhibit it against *S. aureus* occurs at concentrations of 900mg / mL, while the fruit extracts of *G. lucidum* are only able to inhibit the growth of *S.aureus* at concentrations of 600mg / mL and 900mg / mL. This occurs due to differences in the number and types of bioactive compounds (secondary metabolites)

produced by the mushroom fruiting bodies in entering, inhibiting, damaging bacterial cell walls, or destroying cells.According toLiu *et al.* (2012), the active compounds that are antibacterial havecapable to inhibit bacterial growth by entering the bacterial cell wall. Antibacterial compounds enter the bacterial cell will be in the vacuole until the structure of the bacteria is abnormal (chage) until finally experiencing lysis (Jung *et al.*, 2008).

When compared with the antibacterial ability of the antibiotics (chloramphenicol) as a positive control used in this study, it appears that the ability of the fruit extracts of these two species of mushrooms to inhibit the tested bacteria is still far from effective, but has a potential as antibacterial. This is because Chloramphenicol is a broad-spectrum antibiotic whose active compounds have been tested. (The average diameter produced by this antibiotic was 26.35 mm against E. coli and 1.50 mm against S. aureus (Table 1 and Figure 7).

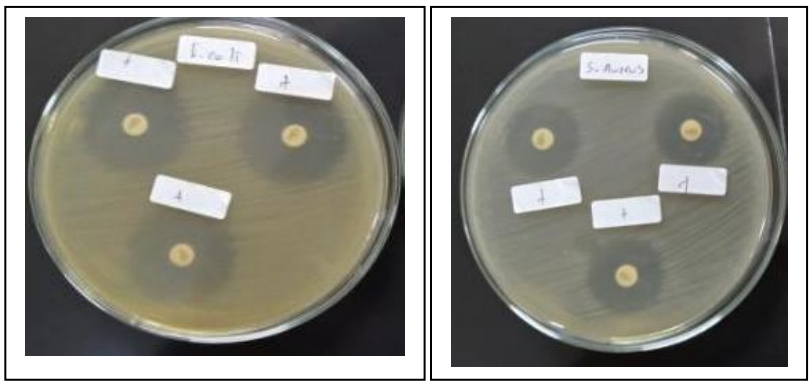

Figure 7.Chloramphenicol Inhibition Zone Against E. coli (A) and S.aureus (B)

Several studies that have been conducted by other researchers using the fruit body extract of *G.lucidum* mushrooms have shown that this mushroom fruit body extract is very effective in inhibiting the growth of tested bacteria, including research Quereshi *et al*. (2010)using methanol extract, acetone extract, and ethanol extract of the fruit body of G.lucidum with a concentration of 40 µg / mL against *E. coli* and *S.aureus*, showed better inhibition zones, namely 16.30 mm, 18.00 and 9.00, respectively. mm against *S. aureus* bacteria and 20,10, 27,40, and 10.60 against *E. coli* bacteria.Antibacterial activity testing conducted by Handrianto and Science (2017)used methanol extract of the mushroom *G. lucidum* with a concentration of 20 $\mu g.ml^{-1}$, 40 $\mu g.ml^{-1}$, 60 $\mu g.ml^{-1}$, 80 $\mu g.ml^{-1}$, and 100 µg . ml^{-1} against S. aureus bacteria, showed inhibition zone activity respectively 8.8 mm, 11.5 mm, 13.4 mm, 15.4 mm, and 17.0 mm.

The same thing also happened to the mushroom fruit body extract of *R. microporus*, where several antibacterial studies that have been conducted by other studies have shown that this mushroom fruit body extract has the ability to inhibit the growth of tested bacteria, including research byFalade et al. (2017)against several types of test microbes including *S.aureus* and *E.coli* bacterias. The results showed that the ethanol extract of *R. microporus* and methanol extract of *R. microporus* with a concentration of 100 mg / ml were able to inhibit the growth of clinical isolates of *E.coli*, *E.coli* ATCC23718, *E.coli* ATTC 35218, *S.aureus* NCB50 with inhibition zones sequentially. based on solvents of 6.73 mm and 4.03; 8.5 mm and 6.27 mm; 7.43 mm and 6.37; 6.63mm and 4.70 mm (diameter of the drag zone excluding the size of the hard disc).

The low ability of the two mushroom fruit body extracts (*G. lucidum* and *R. microporus*) to inhibit the growth of the tested bacteria is likely influenced by the environment where this fungus grows. These two species of mushrooms grow on wood

stumps at the Ragunan campground and the campus yard of National University, Pejaten, PasarMinggu, South Jakarta. The environmental conditions for growing of this fungus are very poor in nutrients and have been polluted. This condition is much different from the environmental conditions in the forest, especially in the highland areas which are rich in nutrients and are not polluted. This greatly affects the nutritional content of mushrooms including the bioactive compounds they produce.

When associated with the concentration of the ethanol extract of the mushroom fruit bodies of *G. lucidum* and *R. microporus* which were used in inhibiting the growth of the two types of tested bacteria, there was no significant increased in the inhibition zone formedmeaning that the addition of the mushroom fruit body extract concentration given did not show an increase in the inhibition zone significantly (Figure 8).

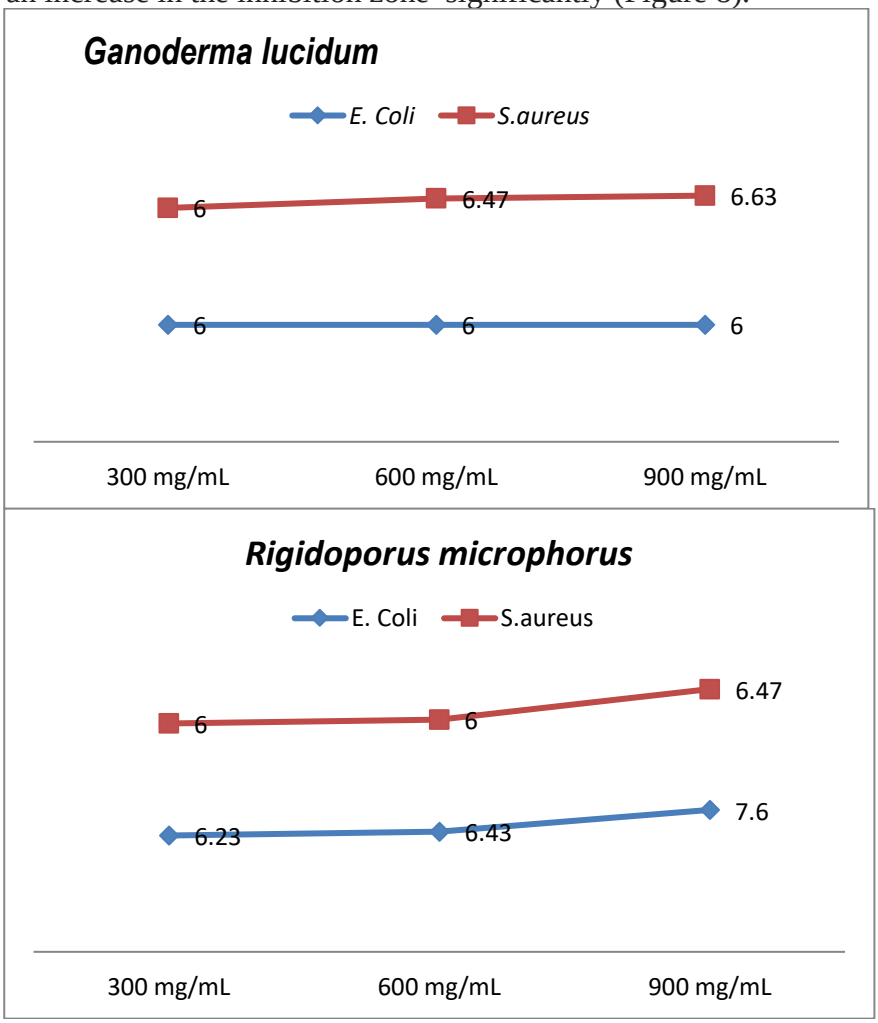

Figure 8: Graph of the Effect of Difference in Concentration of *G.lucidum* and *R.microphorus* Mushroom Extracts on the Growth of both Test Bacteria

Conclusion

Based on the results of research that had been done, it can be concluded that *G. lucidum* extract is only able to inhibit *S. aureus* bacteria. Meanwhile,the *R. microporus* extract was able to inhibit *E.coli* and *S.aureus*. There was no significant increase in the inhibition zone that occurred in the tested bacteria with the addition of the concentration of the mushroom fruit body extract given.

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