111 Lita Meilina/ANTIBACTERIAL ACTIVITY OF Pleurotus ostreatus Grey oyster VARIETY AGAINST PATHOGEN BACTERIAL OF Salmonella typhi

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ANTIBACTERIAL ACTIVITY OF *Pleurotus ostreatus* Grey oyster VARIETY AGAINST PATHOGEN BACTERIAL OF *Salmonella typhi*

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Abstract

Salmonella typhi was one of pathogen microbial which can be prevented granting both synthetic or herbal antimicrobial substances, e.g from an Pleurotus ostreatus species Grey oyster variety that was one of an improvement strain from Pleurotus ostreatus and Pleurotus sajorcaju species. This research aimed to know the extract activities of Pleurotus ostreatus Grey oyster variety as antimicrobial agent against Salmonella typhi. This research used True Experimental with Postest-Only Control Design which were done by in vitro Diffusion methods. The materials which used were Pleurotus ostreatus Grey oyster variety and a panel microorganism of Salmonella typhi. This test was performed at six concentrations of each extract 1.0, 2.5, 5.0, 10.0, 15.0, and 20.0 mg/mL and ethanol control 500 mg/mL. The observation parameters was growth inhibitory diameters (DHP) of Pleurotus ostreatus Grey oyster variety extract as antimicrobial agent against Salmonella typhi. This finding of antibacterial activities by diffusion method was confirmed best inhibition zone in 2.5 mg/mL showed diameters about 22,62±0,990 mm. Parametric analyze of One-way ANOVA results test showed significant differences in inhibition zone diameters between groups. Test results of LSD and Duncan showed significant differences at 5.0 mg/mL, 20.0 mg/mL and K500.0 mg/mL to the others concentrations. From the statistical test results obtained a conclusion that the extract showed a good influence as antibacterial agent against Salmonella typhi.

Keywords : *Pleurotus ostreatus Grey oyster variety, Salmonella typhi*

INTRODUCTION

Salmonella typhi is a gram negative bacterium which caused enteric fever and pathogenic in humans. Approximately 420.000 deaths occur annually in Asia due to typhoid fever. Without treatment, case-fatality rates of infection are 10%. With appropiate antibiotic therapy treatment, case-fatality rates can be reduced to below 1%. Although the infection is treatable with antibiotics, treatment is complicated by growing resistance to widely available oral antibiotics in several areas of the world, including Southeast Asia [15].

The using of antibiotics to treat the disease which is caused due to bacterial infection, can cause some problems related to the toxic effects of drugs, drugs residues and the growing of resistance microbes. Antimicrobial resistance has become a global problem. Strategies to improve the current situation include research in finding new and innovative antimicrobials [4].

New antimicrobial production from bio-pharmacy (medicinal plants) is an alternative to consider for use [10]. This research use oyster mushroom because this species can grow widely in tropic and sub-tropic area, it is easily cultivated as well. There are approximately 40 oyster mushroom species and ranked in the second grade among the other important cultivated mushrooms in the world [2].

The oyster mushrooms contain pleuran compound which act as antitumor, lowering cholesterol levels and antioxidant. The presence of polysaccharide, especially ß-d-glucan give positive effect as antitumor, anticancer, antivirus (icluding AIDS), lowering cholesterol levels, antifungal, antibacterial and can boost the immune system [3].

Due to oyster mushroom potential which give many benefits, many researchs are explored to create new oyster mushroom varieties, one of the results strain is Grey oyster variety which is improvement strain from Pleurotus ostreatus and Pleurotus sajorcaju [11]. This species has advantages in wider shape, thick cowls and duratibility outside of cooling machine (refrigerator) keep it fresh for up to 5 days [12].

According to the antibacterial activities testing of Pleurotus ostreatus and Pleurotus sajorcaju, both of the species showed as antimicrobial agent. Pleurotus ostreatus has been explored to against simple and multiple bacteria isolates of Eschrichia coli, Staphylococcus epidermidis, S. Aureus [1] and species of Candida that has been resistance of drugs [14]. Pleurotus sajorcaju has substansial

that proved as antimicrobial, anti hypercholesterolemia, antioxidant and toxicity activities to Hep-2 cancer cell [9].

The antimicrobial exploration from Pleurotus ostreatus Grey oyster strain has not been exposed. Therefore this research is designed to test the antibacterial activity against phatogen microbe of Salmonella typhi.

MATERIALS AND METHODS

Mushroom collection

Oyster mushrooms Grey oyster variety were collected from mushrooms warehouse of IKIP PGRI Jember, Indonesia working with Agathapratama-Group. Mushrooms were initially dried in an dehumified room, then further dried in an oven at ca. 40°C and finely powdered.

Preparation ef Extract

175 grams of mushrooms powder were extracted with 3,5 L of 96 % solvent of Ethanol using maceration apparatus [8]. The residue was filtered and concentrated to a dry mass in a rotary evaporator (rotavapor) at 40°C and 200 rpm. The fitrate thus obtained was used as mushroom extract. The concentrated extract was re-dissolved in aquadest which helped dissolved firstly by Twin solvent into 1.0 , 2.5 , 5.0 , 10.0 , 15.0 , 20.0 mg/mL extract concentration [13].

Test Microorganism

The Gram-negative bacteria which used were collected from Bio-Pharmacy Laboratory, Pharmacy Faculty of Jember University, Indonesia. The bacterial strains were maintained in NB (Nutrient broth). For antibacterial test, organism were grown overnight in NB medium followed by incubation at 37°C [7].

Antimicrobial Susceptibility Testing

The antimicrobial activities were found by using agar diffusion technique in petriplates. 0.5 mL of the seeded broth containing 10^4 test organism was inoculated uniformly. Briefly 5 mL of each extract concentration was loaded on a sterile filter paper disc 14.20 mm in diameter and air dried. Indicator organisms were spread on Nutrien Agar plates with sterile effusion and the disc were placed on agar plates. After incubation for 48 hours at 37°C, a clear zone around a disc was evidence of antibacterial activity. Diameter of the zones of inhibition was measured in millimeters. Each test was prepared in quarduplicates and solvent of ethanol was used as a negative control [7].

Statistical Analysis

All experiments were conducted in quadruplicates and the parameters were given as means ± standard deviation using statistical package within Microsoft® Excel Version 2010. And parametric analysis of DHP on each groups using SPSS 17.0 Version to analyze the different inhibition zones between groups [6].

RESULTS AND DISCUSSION

Inhibition zone of Pleurotus ostreatus Grey oyster extract and ethanol as control against Salmonella typhi showed various diameter (Figure 1). The extract of Pleurotus ostreatus Grey oyster in 2.5 mg/mL was more potent and revealed high zone (22.62±0,990 mm) formation against Salmonella typhi, whereas the other concentration of extract showed not so different in diameters. The results of present study revealed that Pleurotus ostreatus Grey oyster extract demonstrated higher antibacterial activity comparison of ethanol control.

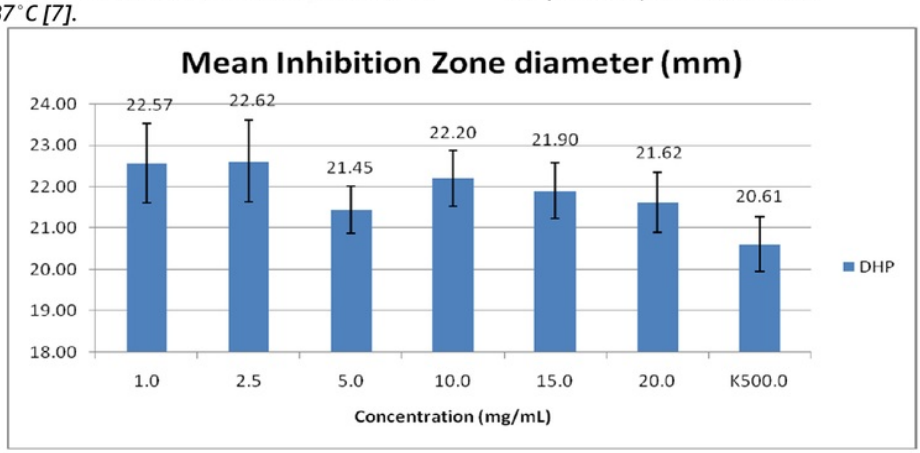

Figure 1. Means inhibition zone diameter (mm) of *Pleurotus ostreatus* Grey oyster variety against *Salmonella typhi*

Table 1.Statistical analysis with One-way Anova

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11.868	6	1.978	3.357	.018
Within Groups	12.372	21	.589		
Total	24.240	27			

Parametric analysis with One-way Anova shows Sig. value at 0.018>0.05 which was categorized there were significant different between groups of Pleurotus ostreatus Grey oyster extract against Salmonella typhi.

Table 2.Post-hoc notation data of DHP Salmonella typhi

Concentration(mg/mL)	Means (mm)
1.0	22,57±0,964[b]
2.5	22,62±0,990[b]
5.0	21,45±0,570[ab]
10.0	22,20±0,675[b]
15.0	21,90±0,674[b]
20.0	21,62±0,729[ab]
K500.0	20,61±0,669[a]

Post-hoc test results showed significant differences at 5.0 mg/mL, 20.0 mg/mL and K500.0 mg/mL to the others concentrations that indicated by differences subset coloumn which were described on alphabetical varians of means coloumn. According to the test results could be concluded that higher concentration of extract, diameter means decreases. This condition was caused the bacteria being resistance of Pleurotus ostreatus Grey oyster extract. The lowest means diameter showed at 5.0 mg/mL concentrations. An error occurred when test of antibacterial activity can also affect the formation of growth inhibitory zone.

Ethanol was taken as negative control for its selective, netral and non-toxic. Fungi and bacteria were difficult to grow on ethanol with a concentraton above 20%. The absorption of ethanol for simplicia compounds was good [5]. Ethanol control showed broad-spectrum (20,61±0,669 mm) at 500 mg/mL that aquivalent with 50% concentration. Compare to this, Pleurotus ostreatus Grey oyster extract also shows broad-spectrum antibacterial activity with a concentration below 20% which indicated its activities better though with low concentration.

Antibacterial activities could be classified based on the magnitude of the inhibitory zones [16].

Table 3.Classificasions of Antibacterial activity

Antibacterial activity	Inhibitory zones (mm)
Weak	<5
Moderate	5-10
Strong	10-20
Very strong	>20

The Pleurotus ostreatus Grey oyster extract concentration ranging from 1.0 – 20.0 mg/mL has diameter means of the inhibitory zone 21.45 - 22.62 mm, so it belongs to very strong activity.

The effectivity of Pleurotus ostreatus Grey oyster extract as antibacterial suspected because of the glycosides compounds like lectins, polysaccharides, polysaccharide-peptide, polysaccharide-protein complex which have been isolated from its stem mushroom and these compounds have been found to have antioxidant, anticancer, antimicrobial, antidiabetic, anti hypercholestrolemic and immunomodulatory properties. The observed phenolic and tannin constituents of P. ostreatus may also elicit antibacterial activity as found in many medicinal plants with mechanisms of action characterized by cell memberane lysis, inhibition of protein synthesis, proteolytic enzymes and microbial adhensin. Whereas, fungal cell wall are rich in non-starch polysaccharides, of which β-glucan are most interesting functional components and phenolic compounds such as protocatechuic acid, gallic acid, homogentisic acid, rutin, myrictin, chrysin, naringin, tocopherol like α-tocopherol and γ- tocopherol, ascorbic acid and β-carotene of each having their own outstanding medical effects [3]. In conclusion, bioactive compounds from Pleurotus

ostreatus Grey oyster extracts could be used as an alternate to antibiotics, considering the side effect and escalating levels of antibiotic resistance among microorganism.

CONCLUSION

Pleurotus ostreatus Grey oyster extract demonstrated high antibacterial activity on Salmonella typhi. The extract was more effective than control ethanol to against pathogenic microorganism studied. Further work is needed to isolates the secondary metabolites and study of metabolic interchanges in bacterial metabolic pathways when applying this extract. It also need to use positive control of traditional antibiotic to evaluate extract efectivity along antibiotics, so the study can explore resistance bacteria. Study of extract toxic effects also needs to be done through toxicity test. The obtained results may be useful for evaluating substances of interest produced by these bacteria as antibacterial agents.

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