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Effect of White Oyster Mushroom (*Pleurotus ostreatus)* Ethanol Extract *on Bacillus subtilis* Bacteria

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ABSTRACT

The increasing number of infectious diseases caused by bacteria, fungi, viruses and parasites in Indonesia, and one of the bacteria that causes this disease is Bacillus subtilis. Therefore, the role of antibacterial is very important for the treatment of this disease. One of the antibacterials from nature is the white oyster mushroom (Pleurotus ostreatus). The purpose of this study was to determine the effect of various concentrations of white oyster mushroom (Pleurotus ostreatus) extract which was effective as an antibacterial against Bacillus subtilis. The research method uses the paper disc diffusion method. The results showed that the bacterial inhibition zone at a concentration of 20 g/ml obtained an average of 9.5 mm in the medium category, a concentration of 40 g/ml obtained an average of 11.3 mm in the strong category, a concentration of 60 g/ml obtained an average of 13, 3 mm in the strong category, a concentration of 80 g/ml obtained an average of 15.5 mm in the strong category and a concentration of 100 g/ml obtained an average of 17.3 mm in the strong category. The conclusion of this research is white oyster mushroom can be used as an anti-bacterial for Bacillus subtilis.

Keywords : Mushroom; White Oyster; Pleurotus ostreatus; Inhibition Zone; Bacillus subtilis

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INTRODUCTION

People are now very familiar with the white oyster mushroom (*Pleurotus ostreatus*) because it tastes delicious and nutritious, high in protein and low in fat. Other benefits of oyster mushrooms are antibacterial and anti-tumor, so oyster mushrooms are also often used to treat various diseases such as diabetes, liver and other diseases. Fresh oyster mushrooms have 80-82.20% moisture content, 0.77-3.6% ash content, and 10.5-30.4% protein content[1].

Bacillus subtilis is a gram-positive bacterium, and this bacterium usually causes meningitis, endocarditis, eye infections, etc [2] *Bacillus subtilis* is found in the human digestive tract, such as the intestine, where excessive bacteria can cause diarrhea and spread through food and drink contamination [3].

The antibacterial active compounds of oyster mushrooms are phenolic compounds, tannins, saponins, flavonoids, steroids and terpenoids [4]. This fungus has antibacterial herbal properties and works by inhibiting the growth of several bacteria (*Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Bacillus subtilis*) [4 5].

The purpose of this study was to determine the effect of various concentrations of white oyster mushroom (*Pleurotus ostreatus*) extract which was effective as an antibacterial against one of the disease-causing bacteria, namely *Bacillus subtilis* using disc diffusion (disc).

METHOD

The materials used were white oyster mushroom (Pleurotus ostreatus) from Mushroom Cultivation in Drenges Sugihwaras Village, Bojonegoro, East Java, Aquadest, Ethanol, Nutrient Agar (NA), pure culture of Bacillus subtilis and this research was conducted in the microbiology laboratory of Maarif Hasyim Latif Sidoarjo University.

The tools used are test tubes, test tube racks, wire loops, 10 ml volume pipette, dropper pipette, burnt spirit, petri dish, analytical balance, micropipette, measuring cup, measuring flask, beaker glass, horn spoon, stirring rod, watch glass. , disc paper, filter paper, funnel, autoclave, incubator, maceration, grinding machine, stove, object glass, aluminum foil, plastic wrap, tissue, cloth, stationery, and sterile glass bottles.

Procedure

Extraction

50 grams of oyster mushrooms were soaked in 150 ml of ethanol solvent at room temperature for 3 days. The maceration solution was centrifuged every 24 hours, the supernatant was filtered using fine filter paper and the filtrate was stored. The residue is immersed again in the same solvent. The resulting filtrate is combined and concentrated in a rotary evaporator to obtain a concentrated extract. The concentrated extract was then tested for its antimicrobial and antioxidant activity.

Antimicrobial Activity Test

The test microorganism used in this study was Bacillus subtilis. Prior to use, Bacillus subtilis was cultured first by inoculating nutrient agar medium, which was made by scraping each bacterium into an inclined agar medium (NA) using a sterile loop needle, then incubated at 37°C. Bacteria grow for 18-24 hours. Antibacterial activity was tested using the paper disk method. Place the paper disc on nutrient agar (NA) medium in a petri dish that has been inoculated with the test microorganism, then drop the sample onto the disc with concentrations of 20 g/ml, 40 g/ml, 60 g/ml, 80 g/ml and 100 g/ml. . Place 5 paper disks with a diameter of 6 mm on the agar media. Different concentrations of oyster mushroom extract were added dropwise to each replicated disc 6 times previously. Incubation for 24 hours in an incubator 33 °C. Observation of the clear zone, measured using a caliper. The research was conducted at the Surabaya Pharmacy Academy Laboratory.

Data analysis

The data can be analyzed using SPSS 22 statistics by comparing the diameter of the inhibition zone from the concentration of each extract of white oyster mushroom (Pleurotus ostreatus) using the Oneway Anova test.

RESULTS

Table 1. Results of Antibacterial Activity Test for White Oyster Mushroom Extract (*Pleurotus ostreatus*) against *Bacillus subtilis* bacteria

Replication	Negative Control	Concentration				
		20	40	60	80	100
1	-	9,5	11,2	13,4	15,7	17,2
2	-	9,5	11,4	13,4	15,9	17,2
3	-	9,6	10,9	13,8	15,2	17,4
4	-	9,4	11,1	12,9	15,3	17,5
5	-	9,7	11,4	13,2	15,3	17,2
6	-	9.4	11,8	13,3	15,5	17,3
Average	-	9,5	11,3	13,3	15,5	17,3
Category	-	Moderate	Strong	Strong	Strong	Strong

Based on the results of testing the antibacterial activity of *Pleurotus ostreatus* extract against *Bacillus subtilis* using 6 replications, the results obtained at a concentration of 20 g/ml an average of 9.5 mm and at a concentration of 40 g/ml an average of 9.5 mm. The concentration of 60 g/ml averaged 13.3 mM, the concentration of 80 g/ml averaged 15.5 mM, and the concentration of 100 g/ml averaged 17.3 mm.

From Table 1 it can be seen that the average diameter of the inhibition zone is 17.3 mm at a concentration of 100% which is the strong category of 17.3 mm, and the average diameter of the minimum inhibition zone is 9.5 mm at a concentration of 20% which is a medium category. . This means that the antibacterial activity of white oyster mushroom (oyster mushroom) extract *against Bacillus subtilis* was most effective at a concentration of 40%, because on average it was a strong category.

Based on the results of statistical tests that have been carried out, it can be concluded that the extract of white oyster mushroom (*Pleurotus ostreatus*) has antibacterial activity which is assessed by testing the presence of an inhibition zone for the growth of Streptococcus mutans bacteria. The mean diameters of the obtained inhibition zones were classified according to [6] in Table 2.

Table 2. Categories of antibacterial activity [6]

Antibacterial Activity	Inhibition Zone Diameter (mm)
Weak	<5
Moderate	5-10
Strong	10-20
Very Strong	>20

Based on these categories, it showed the inhibitory power of white oyster mushroom *(Pleurotus ostreatus*) against *Bacillus subtilis* bacteria at a concentration of 20% in the medium category and at a concentration of 40-100% in the strong category. The antibacterial activity which was classified as moderate-strong at each concentration of white oyster mushroom (*Pleurotus ostreatus*) extract was thought to be due to the presence of saponin compounds. The mechanism of action of saponins as antibacterial is the inhibition of bacterial colonization[7].

DISCUSSION

The first mechanism is the breakdown and destruction of the cell wall, followed by the destruction of other components, which kill the bacteria. Saponins, flavonoids, and terpenoids work by changing the permeability of the cell wall, causing the cell membrane to rupture. Furthermore, phenolic compounds, tannins and alkaloids cause damage by interfering with enzymatic processes in cells [8]. Saponins interfere with cell membrane permeability by producing foam, which results in the release of enzymes and proteins from cells [8]. Phytochemical test results show that Rubber Seed Bark Extract Only Contains saponins. saponinsis an antibacterial compound that can causes proteins and enzymes to in cells [9]. In addition, saponins also inhibit the protein synthesis stage, where protein synthesis is an important metabolic process in cells, which is related to the survival and replication of bacteria, causing total cell damage and bacterial cell lysis [10]. Flavonoid compounds, especially catechins, have been extensively studied for their antibacterial properties in Gram-negative and Gram-positive bacteria over the years. Two mechanisms are involved in the interaction of flavonoids and the lipid bilayer [11], and flavonoids have been shown to act by binding to the lipid bilayer and by inactivating or inhibiting the synthesis of intracellular and extracellular enzymes [12].

Terpenoids are known to be effective in inhibiting bacterial growth. The antibacterial mechanism of terpenoids is the breakdown of membranes involving lipophilic components [13]. Triterpenoids interfere with transmembrane proteins (called porins) in the outer membrane of bacterial cells, with the consequence of disrupting cell wall permeability and reducing cell nutrition, leading to bacterial cell death [14]. The findings of [15] showed that terpenoids cause cell death because cells lose membrane integrity

and function. Alkaloids are compounds with various structures that have been shown to have antibacterial activity (such as quinolones, metronidazole, or others) through inhibition of enzymatic activity or other mechanisms [16].

The mechanism of inhibition of phenolic compounds is through enzymatic inhibition. This inhibition occurs by reacting with sulfhydryl groups on proteins [17]. When extracellular proteins are damaged, hydrogen bonds cannot maintain cell wall and membrane permeability. This decrease in permeability results in an imbalance of macromolecules and bacterial cell lysis [18].

The biological activity of tannins may be related to their oxidation and aggregation patterns. Tannins have an antibacterial effect through the mechanism of protein complexing through covalent and non-covalent interactions [17].

CONCLUSIONS

Based on the results of research and data analysis, it can be concluded that there is antibacterial activity of white oyster mushroom extract against *Bacillus subtilis* bacteria. The antibacterial activity of white oyster mushroom (*Pleurotus ostreatus*) extract against *Bacillus subtilis* was the most effective at a concentration of 40% with an average inhibition zone of 11.3 mm with a strong category.

REFERENCES

1. Lisa, M. *Et Al. Pengaruh Suhu Dan Lama Pengeringan Terhadap Mutu Tepung Jamur Tiram Putih (Plaerotus Ostreatus)*. *Jurnal Keteknikan Pertanian Tropis Dan Biosistem* Vol. 3 (2015).
2. Rahmawati, F. & Bintari, S. H. Studi Aktivitas Antibakteri Sari Daun Binahong (Anredera Cordifolia) Terhadap Pertumbuhan Bacillus Cereus Dan Salmonella Enteritidis. *Life Science* **3**, 103–111 (2014).
3. Wajdi, S. A., Kasmiyati, S. & Hastuti, S. P. Uji Aktivitas Antibakteri Campuran Ekstrak Biji Kelor (Moringa Oleifera) Dan Daun Kersen (Muntingia Calabura) Terhadap Pseudomonas Aeruginosa Dan Bacillus Subtilis. *Journal Of Tropical Biodiversity And Biotechnology* **2**, 10 (2017).
4. Sukmawati Ika K, B. S. S. N. R. S. Aktivitas Antibakteri Ekstrak Tiram Putih (Pleurotus Ostreatus) Terhadap Staphylococcus Aureus, Escherichia Coli, Pseudomonas Aeruginosa, Dan Bacillus Cereus. *Journal Of Pharmacopolium* **2**, 63–67 (2019).
5. A'yunin A Q, N. R. And P. A. S. *Pengaruh Tongkol Jagung Sebagai Media Pertumbuhan Alternatif Jamur Tiram Putih (Pleurotus Ostreatus) Terhadap Aktivitas Antimikroba*. (2016).
6. Davis, W. W. & Stout, T. R. *Disc Plate Method Of Microbiological Antibiotic Assay I. Factors Influencing Variability And Error1*. *Applied Microbiology* (1971).
7. Khairani, K. *Uji Aktivitas Antibakteri Ekstrak Jamur Tiram Purih (Pleurotus Ostreatus) Terhadap Bakteri Streptococcus Mutans Penyebab Karies Gigi*. *Jurnal B-Dent* Vol. 4.
8. Godstime, O. C., Felix, E. O., Augustina, J. O. & Christopher, E. O. *Mechanisms Of Antimicrobial Actions Of Phytochemicals Against Enteric Pathogens-A Review*. *Journal Of Pharmaceutical, Chemical And Biological Sciences* Vol. 2 Http://Www.Jpcbs.Info (2014).

9. Fidyandini, H. P. & Silviana, L. *Uji In Vitro Aktivitas Antibakteri Ekstrak Cangkang Biji Karet Dan Biji Karet Terhadap Aeromonas Hydrophila In Vitro Test Of Antibacterial Activity Of Shell And Rubber Seed Extracts Against Aeromonas Hydrophila*. Vol. 6.

10. Purwanti, F. *Et Al.* Efektivitas Antibakteri Ekstrak Lichen Parmelia Sulcata Terhadap Pertumbuhan Bakteri Shigella Dysenteriae Dan Bacillus Cereus Antibacterial Effectivivess Of Extract Of Lichen Parmelia Sulcata On The Growth Of Bacterial Shigella Dysenteriae And Bacillus Cereus.

11. Tsuchiya, H. Membrane Interactions Of Phytochemicals As Their Molecular Mechanism Applicable To The Discovery Of Drug Leads From Plants. *Molecules* **20**, 18923–18966 (2015).

12. Reygaert, W. C. The Antimicrobial Possibilities Of Green Tea. *Frontiers In Microbiology* Vol. 5 (2014).

13. Bobbarala, V. *A Search For Antibacterial Agents.* (2012).

14. Rahmawati, F. & Harnina Bintari, S. Studi Aktivitas Antibakteri Sari Daun Binahong (Anredera Cordifolia) Terhadap Pertumbuhan Bacillus Cereus Dan Salmonella Enteritidis Info Artikel. *Unnes J Life Sci* **3**, (2014).

15. Zacchino, S. A. *Et Al.* Plant Phenolics And Terpenoids As Adjuvants Of Antibacterial And Antifungal Drugs. *Phytomedicine* **37**, 27–48 (2017).

16. Othman, L., Sleiman, A. & Abdel-Massih, R. M. Antimicrobial Activity Of Polyphenols And Alkaloids In Middle Eastern Plants. *Frontiers In Microbiology* Vol. 10 (2019).

17. Coppo, E. & Marchese, A. Antibacterial Activity Of Polyphenols. *Current Pharmaceutical Biotechnology* **15**, 380–390 (2014).

18. Kusumaningrum Irma K. *Isolasi Dan Identifikasi Kandungan Senyawa Kimia Pada Parmotrema Tinctorum (Despr Ex. Nyl.) Hale Dan Hypotrachyna Osseoalba (Vain.) Y.S. Park & Hale Serta Uji Bioaktivitasnya Sebagai Senyawa Sitotoksik Dan Antioksidan*. (2011).