An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

ANTIBACTERIAL ACTIVITY OF *EXTRACT MAGIC MUSHROOM (PSILOCYBE CUBENSIS* (EARLY) SINGER*)* AGAINST*STAPHYLOCOCCUS AUREUS, ESCHERICHIA COLI,* AND *BACILLUS CEREUS*

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ARTICLE INFO

Key Words

Magic mushroom (Psilocybe cubensis), antibacterial, *Staphylococcus aureus*, *Scanning Electron Microscope*

ABSTRACT

Infection caused by bacteria is one of the problems in the health sector which has a high prevalence and often occurs in tropical countries such as Indonesia. Generally, a lot of infections are treated with antibiotic therapy, but in practice there are many cases of resistance that encourage development in alternative medicine using natural ingredients. One of the plants suspected of having antibacterial activity is Magic mushroom (*Psilocybe cubensis*) because it consists of psilocin and psilocibin which are included in the alkaloid group and have been reported widely as antibacterial. The aimed of this research for knowing antibacterial activity of the magic mushroom extract with broth microdilution methode and scanning electron microscope. Magic mushroom showed activity against *Escherichia coli, Staphylococcus aureus*, and *Bacillus cereus* with consecutive MIC 8.192 ppm, 16.384 ppm and 8.192 ppm. The test result in SEM (Scanning Electron Microscope) using 4 times concentration of MIC showed decreasing size of *Bacillus cereus* and *Escherichia coli* cells.

INTRODUCTION

Indonesia is a tropical country that has a high prevalence of infectious diseases.This bacterial disease is usually treated with antibiotics such as chloramphenicol, dicacyclin and methicillin, but it is important to note that excessive use of antibiotics and antibiotics for long periods can cause resistance to bacteria. Danger of resistance of bacteria and medical costs are quite high, raising awareness to find alternatives to antibiotics by using traditional medicines derived from plants as alternative medicines against bacterial infections (Wise, 2003). Search for new anti-bacterial sources by conducting Research from natural materials. *Psilocybe cubensis* or commonly known as magic mushroom is a group of mushrooms made from natural ingredients that are hallucinogenic, including in the genus Psilocybe. This fungus has a bald head covering and lives in humid areas such as fungus in general, around herbivorous animal feces such as cattle, horses, buffalo, and others (Suaniti, 2018). Psylocybe cubensis is an alkaloid-derived fungus that contains psylosibine, psylosin, baeocystin compounds (Novitaloka, 2013). Besides alkaloids arereported widely as antibacterial becauseits mechanism of action by

disrupting the constituent components of peptidoglycan on bacterial cells, so that the cell wall layer do not formed intact and causes cell death. Other mechanisms of antibacterial alkaloids are alkaloid components which are known as DNA interkeators and inhibit bacterial topoisomerase enzymes (Darsana, 2012).Infectious diseases caused by bacteria and fungi in developing countries likeIndonesia are still the highest contributor to morbidity and mortality. It is still a big problem and a serious challenge for Indonesia to find alternative antibiotic treatment in order to reduce the high incidence of the disease (Utami, 2005). Infectious diseases are caused by several microorganisms such as bacteria, viruses, parasites, and fungi that enter and multiply in the body (Jawetz et al., 2005). There are several types of pathogenicbacterial and fungal thatinfect humans be able to including*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans* (Leboffe, 2011). Based on the description above, this study was conducted to determine the antibacterial activity of magic mushroom (*Psilocybe cubensis*) against *Stahpylococcus aureus, Escherichia coli,* and *Bacillus cereus* bacteria. This can be an alternative treatment for infectious diseases caused by bacteria and fungi.

MATERIALS AND METHODS

Tools and Materials:

The tools were used in this study were petri dish, erlenmeyer, sterile scissors, incubators, ose needle, ruler, sterile knife, centrifugator, shaker, test tube, tube rack, spiritus burners, analytical balance, pipette, spatula, glass beaker, measuring cup, stirring bar, bath, autoclave, fatty cotton, yarn mattress, micropipette, LAF, tip, heat-resistant plastic, sterile tissue. The materials used in this study were 70% alcohol, comparative antimicrobials (Tetracycline), distilled water sterile, Magicmushrooms (*Psilocybe cubensis*),nutrient agar, nutrient broth,

Bacteria samples were used in this study were *Escherichia coli, Bacillus cereus* and *Staphylococcus aureus.*

Method:

This research was experimental, which was done by testing the magic mushroom extract on the bacteria *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus cereus.* The antibacterial activity of the magic mushroom is determined through several stages, from collecting materials in the Bandung, West Java. Then the material is determined, the mushroom processing starts from wet sorting, dry sorting to extracting. Furthermore, sterilization of tools and materials using an autoclave. Then rejuvenation of the test bacteria and making a bacterial suspension with the concentration is needed in the study. Fungal extract testing was carried out singly on the bacteria *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus cereus* with the method used was microdilution to determine the value of Minimum Inhibitory Concentration (MIC) and Minimum Kill Concentration (KBM). The next step to be more convincing of the antibacterial activity in these psilocybe fungi is to observe the changes in fungal morology using the Scanning Electon Microscopy (SEM) method.

RESULTS AND DISCUSSION

This research begins with the process of finding magic mushroom samples obtained in Bandung, West Java. In that area, a lot of magic mushrooms are found from cattle dung owned by residents, with a favorable environment for the growth of magic mushrooms so that their availability is quite good.Making simplicia was done by collecting 1.5 kg of magic mushroom. The part that was magic mushrooms body was taken as asample. After getting wet mold, wet sorting, washing, changing the shape are made to reduce the surface area, followed by drying process using oven, dry sorting and storage. Simplisia dried magic mushroom gained 156.15 grams. So the yield of simplicia obtained is 10.41%.

Tests of water soluble extracts and soluble extracts in ethanol aim to determine the amount of active compounds extracted in the solvent from a number of simplisia powders. From the data above, 22.38% based on data consist of water soluble extracts and 7.63% consist ethanol soluble extracts indicate that the compounds contained in the magic mushroom simplicia are more soluble in ethanol compared to water. Test for total ash content and test for insoluble ash content in acid aim to determine minerals and inorganic contamination (Sudarmadji, 1986). The amount of total ash in the magic mushroom extract indicates that an extract containing much or at least minerals is obtained. Based on table 1, the total ash content is 9.55%, showing the presence of insoluble ash content in the acid and the presence of sand or other impurities in low levels. The extraction process is carried out by means of the alkaloid extraction method which is assisted by sonication. This aims to enable the process of withdrawal of active compounds that are more optimal. The solvent used in this maceration process is 10% acetic acid, aqua deion and sodium bicarbonate added to neutralize the pH of the acidic solution due to the addition of acetic acid. Then maserat from this process is filtered then added to chloroform and centrifuged, after centrifuged the bottom layer is evaporated until a thick extract is obtained with a constant weight, the magic mushroom extract obtained is 46.03 grams. So the extract yield obtained is 29.47%. From the results of this yield, it can be seen that the extraction method is effective to obtain a sufficient amount of extract from the limited availability of simplicia. Based on table 2 shows the presence of alkaloids in the extract. Alkaloid has the ability as an antibacterial. The suspected mechanism is to disrupt the constituent components of peptidoglycan in bacterial cells, so that the cell wall layers do not formed intact and cause the death of these cells (Mahanani, 2012). The testingantibacterial activity of the magic mushroom extract was conducted using the Microdilution method. This method can find out the Minimum Inhibitory Concentration (MIC) which is defined as the lowest concentration of a compound that can decrease 80% or more growth compared to negative controls (NCCLS, 2009) and the Minimum Kill Concentration (KBM) which is the lowest concentration that shows no growth (Holetz, 2002). The KHM value of magic mushroom extract against Staphylococcus aureus was 16,384 ppm. Whereas the bacteria *Escherichia coli* and *Bacillus cereus* showed MIC values of 8,192 ppm. The results of the MIC values of tetracycline compared to all antibiotics in the test bacteria were classified as strong where the MIC value was 1 ppm. KBM test results showed that all samples still contained bacterial growth, which means the value of KBM against *Staphylococcus aureus* was at a concentration of> 32,768 ppm. while the KBM value in *Escherichia coli* and *Bacillus cereus* bacteria is> 16,384 ppm. In this study, a *Scanning Electron Microscope* was performed with the aim of seeing morphological changes in the bacterial body structure after exposure to magic mushroom extract. The principle of SEM is image made based on the detection of new electrons (secondary electrons) or reflected electrons that emerge from the surface of the sample when the sample surface is scanned with electron beam (Perdina, et al., 2014). From the SEM results, it showed *Escherichia coli* bacteriawere exposed to the test extract with a concentration of four times the MIC (32,768 ppm) were seen to be damaged where the contents of the bacterial cells underwent lysis. In the SEM results, the *Bacillus cereus* bacteria showed changes in bacterial cells in the form of shrunken cells. Changes in bacterial cells exposed to the extract are caused by the presence of antibacterial activity that is thought to originate from the alkaloid compounds contained in the magic mushroom extract. Because these group compounds work by influencing membrane permeability that can inhibit bacterial growth.

Table 1. Simplicia Characterization

No	Test	Results (%)
1	Water- soluble content	22,38
2	Etanol-soluble content	7,63
3	Total ash content	9,55
4	Ash content does not dissolve in acids	4,61

Table 2.Phytochemical Magic mushroom screening results

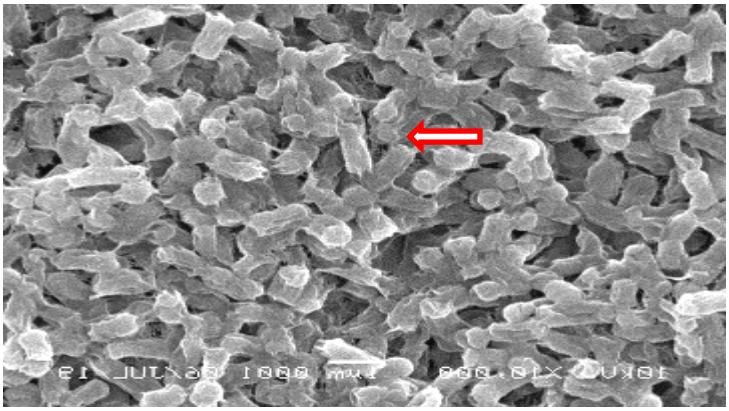
No	Test	Results
1	Alkaloid	+
2	Flavonoid	-
3	Saponin	-
4	Tanin	-
5	Kuinon	-

Information: + = Detected; - = not detected

Tabel 3.The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the Test Bacteria

Bacteria	Extract Test (ppm)		Tetracycline (ppm)	
	MIC	MBC	MIC	MBC
Staphylococcus aureus	16.384	>16.384	1	>512
Escherichia coli	8.192	>16.384	1	>512
Bacillus cereus	8.192	>16.384	1	>512

Information: MIC = Minimum Inhibitory Concentration, MBC= Minimum Bactericidal Concentration



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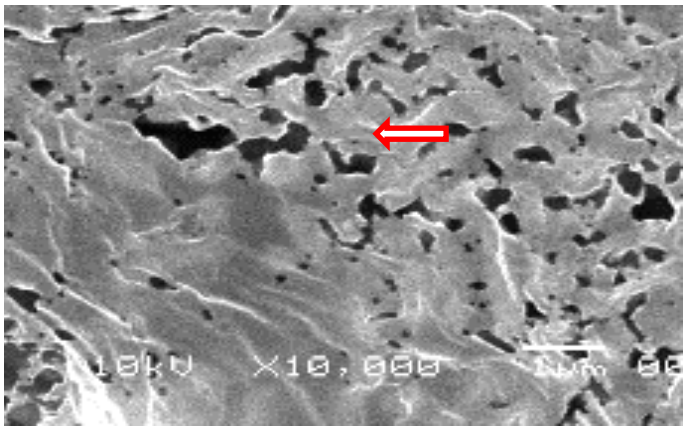


B

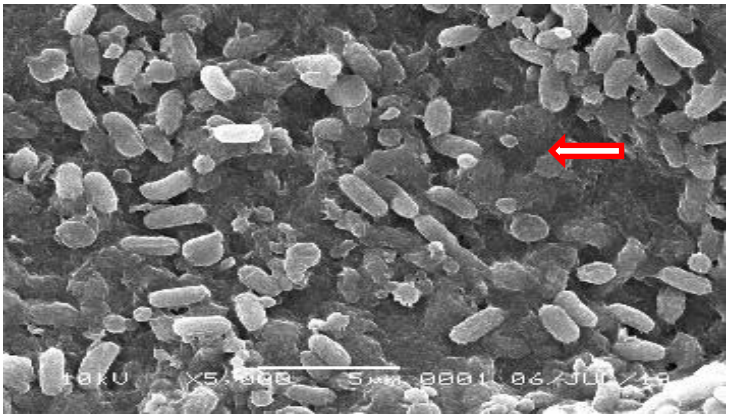
Figure 1.The results of the Scanning Electron Microscopy (SEM) the reactions of the extractof *Psilocibin cubensis*to the infection of *Escherichia coli*

Information:

A = normal *Escherichia coli*; the arrow shows the control cell

B = Bacterial cell damage due to exposure to extracts

A



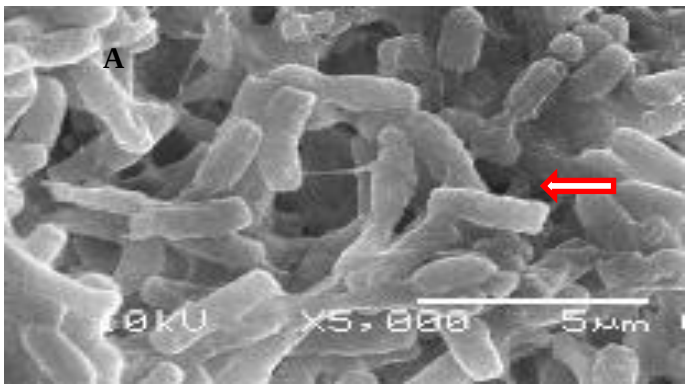


Figure 2.The results of the Scanning Electron Microscopy (SEM) the reactions of the extractof *Psilocibin cubensis*to the infection of *Bacillus ceureus*

B

Information: A = normal *Bacillus ceureus*; the arrow shows the control cell

B = bacterial cells in the form of shrunken cells.

Morphological changes bacteria can be caused by the content of alkaloid compounds, flavonoids, steroids, triterpenoids and tannins, which has antibacterial activity. Alcaloid has a mechanism as an antibacterial by disrupting the constituent components of peptidoglycan on bacterial cells so that the cell wall layer do not formed intact and causes antibacterial activity. cell death. In addition, alkaloids work by disrupting the constituent components of peptidoglycan and inhibiting the enzyme topoisomerase which has a very important role in the process of replication, transcription and recombination of DNA by cutting and connecting single strands or double strands of DNA (Sukmawati,2019)

CONCLUSION

From the results of research on testing the antibacterial activity of Magic mushroom extract (Psylocibin cubensis) it can be concluded that:

- Magic mushroom extract (Psilocybe cubensis (Early) Singer) has antibacterial activity against Staphylococcus aureus, Escherichia coli and Bacillus cereus bacteria.
- Magic mushroom extract with the best MIC value that is 8,192 ppm with KBM value> 16,384 ppm shows the antibacterial activity of Escherichia coli and Bacillus cereus bacteria.
- Value SEM (Scanning Electron Microscope) test results with a concentration of four times the MIC indicate a change in cell size in Bacillus cereus and damage to Escherichia coli cells.

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