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Antibacterial activity of extracts and fractions of wood ear mushroom (*Auricularia auricula*)

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ABSTRACT

Infection is a disease caused by pathogenic bacteria, such as *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, and *Pseudomonas aeruginosa*. The wood ear mushroom (*Auricularia auricula*) has been empirically and scientifically proven as an agent that can treat infections because it contains antimicrobial properties like polysaccharide and protein complex. This study aimed to determine the antimicrobial activity of the *A. auricula* extracts and fractions. The first stage of the test comprised the collection, determination, and processing of the test plant. The *simplicia* was characterized by its water content, ash content, acid solubility, and total ash content. The next step was reflux extraction using 96% ethanol. After a concentrated extract was formed, it was subjected to phytochemical screening and fractionation using liquid-liquid extraction (LLE) with n-hexane, ethyl acetate, and methanol-water solvents. Each of the *A. auricula* extracts and fractions was tested for antimicrobial activity. Microdilution method was employed to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC), which were later compared with the antibiotic properties of tetracycline. Scanning Electron Microscopy (SEM) was used to observe any morphological changes in the tested bacteria. The MICs of the *A. auricula* extract, n-hexane fraction, and ethyl acetate fraction against *S. aureus* were 256 µg/mL, 128 µg/mL, and 64 µg/mL, respectively. However, the bactericidal effects of the test mushroom were not achieved in the experiment. Based on the MICs, the ethyl acetate fraction has the best inhibitory activity. Moreover, the bioautography test of this fraction produced Rf value= 0.78, implying the formation of a zone of growth inhibition. The SEM of the *A. auricula* extracts and fractions proved that this mushroom altered the morphology of *S. aureus* bacteria—i.e., the cells became rounded and tapered and showed indications of cell shrinkage and damage. As a conclusion, the ethyl acetate fraction of *A. auricula* has the best antimicrobial activity against *S. aureus.*

Keywords: *Auricularia auricula*, antibacterial, Scanning Electron Microscopy (SEM)

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INTRODUCTION

In Indonesia, the incidence of infection increases every year. The combination of poor public awareness of hygiene and the tropical climate of Indonesia allows the rapid growth of bacteria, viruses, and fungi. The Basic Health Research in 2013 found that every province in Indonesia had a different prevalence of infection. East Nusa Tenggara is the province with the highest incidence of acute respiratory infections or ARI (41.7%) and pneumonia in children under five years old (38.5%), as well as the highest incidence and prevalence of pneumonia at all ages (4.6% and 10.3%, respectively). West Java has the highest occurrence of pulmonary tuberculosis (TB) (0.7%). Papua has the highest rate and the most extended prevalence period of diarrhea (6.3% and 14.7%, respectively), and Aceh has the highest risk of diarrhea (10.2%) (Kementrian Kesehatan RI, 2013).

Antibiotics are compounds used to treat bacterial infections. However, a problem like inappropriate consumption triggers and increases the lack of sensitivity of pathogenic microorganisms to antibiotics. This antibiotic resistance leads to ineffective treatment, such as in the case of *Staphylococcus aureus*. *S. aureus* is often found as normal bacterial flora in human's skin and mucous membranes, but it can also be a cause of infection in humans and animals. Tissues or organs infected with it often develop into diseases with typical signs, such as necrosis, inflammation, and abscess formation (Aulia *et al.*, 2007). Alternative medicine that can be used to treat its infection is the wood ear (*Auricularia auricula*). It is a species of mushroom from the class Basidiomycetes that contains polysaccharides and protein complexes that possess antitumor, immunomodulatory, cardiovascular, hypercholesterolemic, antiviral, antibacterial, and antiparasitic properties (Wasser and Weis, 1999).

The hot water extract from *A. auricula* exhibits antibacterial activity against *S. aureus* at a concentration of 100 µg/mL, as evidenced by the formation of a 15mm-diameter zone of inhibition in previous research (Udu-Ibiam *et al.*, 2014). A universal solvent like 96% ethanol can attract polar, semi-polar, and nonpolar compounds of the *simplicia*. Therefore, during the fractionation process, it can separate the compounds of *A. auricula* according to their polarity. Aside from screening this mushroom for its antimicrobial activity against both gram-positive and gram-negative bacteria—*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus*, and *Escherichia coli*—a complementary bioautography test can identify which compounds induce this character. This test relies not only on Rf value but also visible changes in cells morphology under a scanning electron microscope after the test bacteria are exposed to the extracts or fractions.

MATERIALS AND METHOD

Materials

Tools: microplate, Petri dish, separating funnel, test tube, Erlenmeyer flask, analytical balance, volumetric paper, micropipette, glass of chemicals, measuring cup, round flask, hot plate, autoclave, incubator, reflux, rotary evaporator, chamber, centrifuge, UV lamp, gel exchange rate, desiccator, tweezers, Laminar Air Flow (LAF), TLC plate GF254, syringe, Eppendorf tube. Materials: 96% ethanol, n-hexane, ethyl acetate, methanol, chloroform, toluene, ether, DMSO, amyl alcohol, aquadest, Dragendorfd's reagent, Mayer's reagent, Liebermann-Burchard reagent, Stiasny reagent, Bouchardat's reagent, magnesium powder, gelatin, H_2SO_4, HCl, $FeCl_3$, NaCl, NaOH, acetic acid, sodium acetate, ammonia, 10% formalin, spirits, aqua pro injection, physiological NaCl, paper discs, cotton, gauze, mattress cord, aluminum foil, cellophane. Media: Mueller-Hinton Agar (MHA) and Mueller-Hinton Broth (MHB). Positive control: Tetracycline.

The wood ear mushroom (*A. auricula*) was obtained from Caringin Bandung Market, which receives supplies from a mushroom cultivation site in Surabaya, East Java. The bacteria used were *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, and *Pseudomonas aeruginosa* obtained from the School of Life Sciences and Technology, Bandung Institute of Technology (ITB), Indonesia.

Methods

The antimicrobial activity test in this study referred to the Clinical and Laboratory Standard Institute (CLSI). The first stage of testing included the collection, determination, and processing of *Auricularia auricula* to identify its antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. The *simplicia* was characterized based on its water content, acid solubility, ash content, and total ash content (Depkes RI, 2008). The second step was reflux using 96% ethanol solvent, followed by extraction in a rotary evaporator until a concentrated extract was formed. Afterward, the extract was subjected to phytochemical screening, which aimed to identify the type of major compounds in *A. auricula*. The next step was fractionation using liquid-liquid extraction method with n-hexane, ethyl acetate, and methanol-water solvents. Each of the *A. auricula* extracts and fractions was tested for antibacterial activity with microdilution method, which determined the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the ethanol extract, n-hexane fraction, ethyl acetate fraction, and methanol-water fraction for *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, and *Pseudomonas aeruginosa*. Tetracyclines, which have a broad spectrum, were used for comparison (CLSI, 2012).

The extract or fraction with the best antibacterial activity was subjected to a bioautography test. Contact bioautography is done by placing the chromatogram plate resulting from the elution of the compound to be tested on solid media that has been inoculated with the test microbes. The presence of antimicrobial compounds is characterized by the presence of clear areas that are not overgrown with microbes (Kusumaningtyas, 2008). The antibacterial activity was marked by a clear circular zone formed on the media, indicating that the compound(s) with specific Rf values can inhibit the growth of the test bacteria. In the final stage, any morphological changes of the *Staphylococcus aureus* and *Escherichia coli* cells after exposure to the *A. auricula* extracts and fractions were observed during the Scanning Electron Microscopy (SEM).

Results and Discussion

The wood ear mushroom in this study was cultivated in Surabaya, Central Java and, then, processed at Jatinangor Herbarium, Laboratory of Plant Taxonomy managed by the Department of Biology, Faculty of Mathematics and Natural Sciences, University of Padjadjaran. The mushroom was identified by comparing the microscopic and macroscopic forms of *Auricularia auricula* with relevant literature. Afterward, it was authenticated as the wood ear with the scientific name *Auricularia auricula*. The material processing started with wet sorting and washing a total of 20 kg fresh *A. auricula*. Then, it was cut into small pieces and dried under direct sunlight for three (3) days to reduce the water content and create dry *simplicia*. The remaining foreign objects in the dry *simplicia* were removed. This series of processes produced 1.02 kg of *simplicia*. Before screening the *simplicia* for antibacterial activity, it was first characterized based on water content, total ash content, water-soluble extract, and ethanol-soluble content. This test aimed to provide information about *A. auricula* and the quality of the *simplicia*. This data was also useful to achieve safe, efficacious, and high-quality *simplicia* or extracts. Each of the *simplicia* had different characteristics and qualities (Table I) because of variations in the location of *A. auricula* cultivation and the effects of air, temperature, humidity, and types of planting media.

Water content assessment aims to measure the amount of water in the *simplicia*. An excess amount of water will accelerate the growth and induce the decomposition of microorganisms; therefore, controlling the water content can reduce the probability of decay and damage during the storage and processing of the materials. In this study, the water content assessment employed azeotropic distillation using toluene as the solvent. Water-saturated toluene can prevent pseudo results or, in other words, the water content extracted by toluene is solely from the *simplicia*. According to the book of Materia Medika Indonesia (MMI), *simplicia* are safe for consumption if they contain <10% water. This threshold marks the amount of water content at which *simplicia* start to decay during the

storage. The assessment results showed that the water content of the *simplicia* from *A. auricula* was 0.14%.

Table I. *Simplicia* Characterization

Tests	Results (%)
Water content	0.14
Total ash content	2.7
Water-soluble content	5.1
Ethanol-soluble content	2

The total ash content testing aims to provide an overview of the internal and external mineral content. The principle of the test is that the heating of material up to a certain temperature at which the organic compound and its derivatives are destructed and evaporated leaves behind minerals and inorganic elements in the test *simplicia*. In this study, the total ash content of the *simplicia* from *A. auricula* was 2.7%. Testing the content of the extract can provide an initial overview of the number of compounds in the *simplicia*. The principle is dissolving material with a solvent to identify the number of compounds in the *simplicia*. In this study, this test found that the *simplicia* contained 5.1% water-soluble extract and 2% ethanol-soluble extract. In the extract preparation, a total of 900 g of *simplicia* was processed with Heat-Reflux Extraction (HRE). Extraction is the process of transferring a compound from a *simplicia* based on its solubility in a particular solvent. The research used HRE because the compounds in *A. auricula* are most likely to reach thermostable. Also, this method allows more extraction of compounds particularly when the *simplicia* is in direct content with the solvent.

The study used 96% ethanol, a universal solvent that can attract all types of compounds, including polar, semi-polar, and nonpolar, of the *simplicia*. The extraction used a 1:15 ratio of *simplicia* to solvent and lasted for 2.5 hours. It produced a liquid extract that was further concentrated in a rotary evaporator. The extract concentration aims to separate the remaining solvent in a liquid extract and, thereby, produce a viscous extract. The viscous extract acquired from 900 g of the wood ear mushroom's *simplicia* that had been dissolved in 15 L of ethanol 96% was 20.83 g with the oil extraction yield of 2.314%).

Based on the results of the phytochemical screening, the concentrated extract of *A. auricula* contains alkaloids, tannins, and steroids/triterpenoids. This finding is in line with the research conducted by Nwachukwu and Uzoeto (2010) that found alkaloid in the ethanol extract of this mushroom. Also, Hafidh *et al.* (2012) explain that alkaloids and tannins are active compounds that exhibit antimicrobial activities by changing the characteristics of the cell walls of the targeted bacteria. The mechanism of growth inhibition by steroids is related to the lipid membranes and can cause damage to the liposomes of the test bacteria (Shihabudeen and Priscilla, 2010). Meanwhile, triterpenoid is an active compound with antibacterial properties that destroy the cytoplasmic membrane (Ochoa *et al.*, 2013).

Fractionation is a process of separating compounds based on their level of polarity. In this study, the Liquid-Liquid Extraction (LLE) in the fractionation process used three different types of solvents, namely n-hexane (nonpolar), ethyl acetate (semi-polar), and methanol-water with 2:8 ratio (polar). Ten grams of the concentrated *A. auricula* extract was fractionated. This process resulted in liquid fractions that were later concentrated using a rotary evaporator. The outputs of this process were 2.22 g concentrated n-hexane fraction (fraction value= 22.2%), 0.18 g ethyl acetate fraction (1.8%), and 5.76 g methanol-water fraction with a ratio 2:8 (57.6%). These findings show that the *A. auricula* extract contains more polar compounds because a large part of it was dissolved in the methanol-water fraction—a polar solvent. However, it also contained semi-polar and nonpolar compounds that were dissolved in ethyl acetate and n-hexane fractions. The polar compounds are flavonoids, tannins, and

polyphenols (including hydroxyl groups). Alkaloids are semi-polar compounds, whereas steroids are a nonpolar lipid.

Microdilution method was used to screen *A. auricula* for antibacterial activity, as represented by Minimum Inhibitory Concentration (MIC). MIC is the smallest concentration of an antibacterial agent that can prevent the growth of the test bacteria over an extended period, i.e., after incubation for 18-24 hours (Efendi and Hertiani, 2013). The concentrations used in the tests were 4, 8, 16, 32, 64, 128, 256, 512, 1024, and 2048 µg/mL that were all obtained from a 4096 µg/mL parent solution. The *A. auricula* extracts and fractions were dissolved in 5% DMSO (Dimethyl sulfoxide) solvent and, then, diluted using sterile distilled water that was added up to a specified volume. DMSO is a solvent that can dissolve the extract and fraction properly because it has no antibacterial activity and, consequently, it will not affect the test results. The test parameter observed in the microdilution method was the turbidity of both extract and fraction. The lowest concentration that shows inhibition of bacterial growth marked by the presence of clear zones is called KHM. Following the MIC assessment was the determination of the Minimum Bactericidal Concentration (MBC) by replanting the aliquot on the agar with different concentrations, i.e., from the lowest to the highest MIC. MBC is the lowest concentration of an antimicrobial agent that can kill bacteria, which is characterized by the absence of bacterial growth on the agar (Zakiyah *et al*., 2013).

The bacteria used in the test were *Staphylococcus aureus* ATCC 65338, *Escherichia coli* ATCC 9027, *Bacillus cereus* ATCC 11778, and *Pseudomonas aeuroginosa* ATCC 8939, which were grouped into gram-positive and gram-negative bacteria. The standard turbidity was the standard McFarland 0.5 CFU/mL; which is similar to containing 108 CFU/mL of bacteria with an absorbance of 0.08-0.1 measured at a wavelength of 625 nm (NCCLS, 2009).

The antibacterial activity testing with microdilution used three test controls, namely a negative control containing Mueller-Hinton Broth (MHB) in the first column, a positive control consisting of Mueller Hinton-Broth (MHB) media and bacterial suspension in the second column, and tetracycline as a control for comparison. The negative control aims to determine whether the media used in the experiment is contaminated and to compare the clarity of the microdilution test. The positive control seeks to identify whether the test bacteria used in the analysis can grow well on Mueller-Hinton Broth (MHB) media. The control for comparison, which used tetracyclines, aims to validate the microdilution method. Tetracycline was selected as a comparator because it was a broad-spectrum antibacterial ingredient that could inhibit the growth of gram-positive and gram-negative bacteria. Meanwhile, Mueller-Hinton Broth (MHB) and Mueller-Hinton Agar (MHA) were selected as the media in this study because they had high reproducibility per test, as well as low inhibitory compounds that could affect the test results of sulfonamides, trimethoprim, and tetracycline and allow pathogenic bacteria to grow and proliferate. These media were also selected because they had been widely used in antibacterial activity testing (CLSI, 2012).

Table II shows that the *A. auricula* extract and fraction have antimicrobial activity against *Staphylococcus aureus* with different MICs, namely 256 µg/mL for the extract, 128 µg/mL for the n-hexane fraction, 64 µg/mL for the ethyl acetate fraction, and 2048 µg/mL for the methanol-water fraction. They also exhibit antimicrobial activities against *Escherichia coli* and *Bacillus cereus* with MIC >2,048 µg/m. Meanwhile, the antimicrobial activities against *Pseudomonas aeruginosa* are > 2048 µg/mL for the extract, n-hexane fraction, and methanol-water fraction and 2048 µg/mL for the ethyl acetate fraction. However, compared to the *A. auricula* extracts and fractions, tetracycline shows better inhibitory activity against all test bacteria because it has lower MICs, i.e., 4 µg/mL for *Staphylococcus aureus*, 16 µg/mL for *Escherichia coli*, 16 µg/mL for *Bacillus cereus*, and 23 µg/mL for *Pseudomonas aeruginosa*. Based on these results, tetracycline assay can be used as a validating agent in antimicrobial activity screening.

Table II. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the Test Bacteria

Bacteria	E µg/mL)		F.NH (µ(g/mL)		F. EA (µg/mL)		F. MA (µg/mL)		T (µg/mL)	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus	256	>2048	128	>2048	64	>2048	2048	>2048	4	4
E.coli	>2048	>2048	>2048	>2048	>2048	>2048	>2048	>2048	4	16
B.cereus	>2048	>2048	>2048	>2048	>2048	>2048	>2048	>2048	4	16
P.aeruginosa	>2048	>2048	>2048	>2048	>2048	2048	>2048	>2048	32	32

Information: E= Extract, F.NH= n-hexane fraction, F.EA= Ethyl acetate fraction, F.MA= Methanol-water fraction, T= Tetracycline

An extract is considered a strong antibacterial agent if its MIC is <100 µg/mL. If the MIC is within the range of 100-500 µg/mL, then the antibacterial activity is moderate. If the MIC is 500-1000 µg/mL, then the extract has weak antimicrobial activity; and if it is >1000 µg/mL, it means that the extract does not have active antibacterial properties (Holetz *et al.*, 2002). Based on the MIC of the *A. auricula* extracts and fractions against *Staphylococcus aureus, Escherichia coli*, *Bacillus cereus*, and *Pseudomonas aeruginosa*, the ethyl acetate fraction has the best antimicrobial activity against *Staphylococcus aureus* with a MIC of 64 µg/mL—a potent antibacterial agent.

The next test was determining the Minimum Bactericidal Concentration (MBC), which was obtained from the MIC value of the microdilution method that was planted on agar. The test results showed that the *A. auricula* extracts and fractions had bactericidal effects on the test bacteria with MBC > 2048 µg/mL. Meanwhile, the same effects of tetracycline on the test bacteria were represented by different MBCs, namely 4 µg/mL for *Staphylococcus aureus*, 16 µg/mL for *Escherichia coli* and *Bacillus cereus*, and 23 µg/mL for *Pseudomonas auruginosa*.

Bioatugraphy aims to detect active compounds with antibacterial properties. In this method, the Thin-Layer Chromatography (TLC) plate is placed on the prepared agar medium to allow diffusion. The diffusion of groups of active compounds from the TLC plate creates zone of inhibition on the agar medium, indicating the presence of antibacterial activity (Kosworo, 2012). The bioautography test was performed on the ethyl acetate fraction using the mixture of ethyl acetate and n-hexane with a ratio of 7:3 v/v as the mobile phase. This mobile phase was selected based on its capacity, i.e., this phase can separate the compounds in the ethyl acetate fraction of *A. auricula* well.

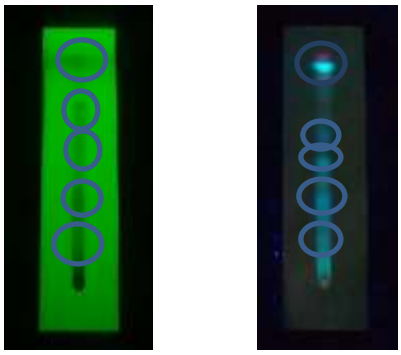

Figure 1. The elution of the ethyl acetate fraction of *Auricularia auricula* as seen under UV light at different wavelengths: (A) 254 nm and (B) 365 nm. The Rf values of the stains or spots are 0.36, 0.61, 0.78, 0.85, and 0.96

As observed under ultra-violet light at 254nm and 365nm wavelengths, the Rf values of the stains of the ethyl acetate fraction were 0.36, 0.61, 0.78, 0.85, and 0.96. To identify which spots represented antimicrobial activity, the TLC plate was subjected to contact bioautography. It was placed on the agar medium for 30 minutes (a direct contact) and incubated for 24 hours at 37°C. The results presented that the spot with Rf= 0.78 showed a zone of growth inhibition on the agar medium, meaning that the compounds of the ethyl acetate fraction have antibacterial properties. To identify which compound owned these properties, the plate was sprayed with the Dragendorff's reagent, Liebermann-Buchard reagent, and $FeCl_3$ to create spotting effects. This process showed that the ethyl acetate fraction (Rf= 0.78) contained tannins and was thereby suspected of having antimicrobial properties. The presence of tannins was indicated by blackish spots or stains on the TLC plate after $FeCl_3$ sprays (Figure 2).

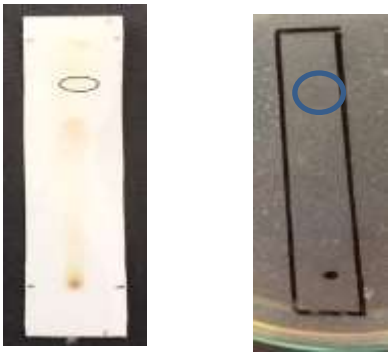

Figure 2. The Results of Bioautography Test of the Ethyl Acetate Fraction of *Auricularia auricula* against *Staphylococcus aureus* (Rf= 0.78)

The final stage, i.e., Scanning Electron Microscopy (SEM), aims to observe morphological changes in targeted bacteria, such as shape, size, and cell walls, after treated with the test extracts and fractions. This test was based on the results of the previous MBC assessment, i.e., that the ethyl acetate fraction and the n-hexane fraction had the best bactericidal effects against the test bacteria.

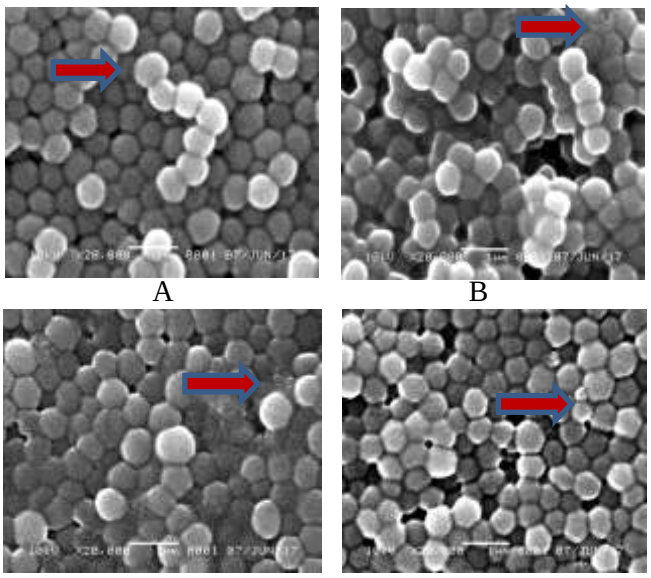

Figure 3. The results of the Scanning Electron Microscopy (SEM), i.e., the reactions of the extract, n-hexane fraction, and ethyl acetate fraction of *Auricularia auricula* to the infection of *Staphylococcus aureus*

Information:
A = normal *S. aureus*; the arrow shows the control cell (20,000x magnification)
B = non-spherical *S. aureus*; the arrow points to a change in shape and damage to the cell walls following the application of the *A. auricula* extract with 8x MIC (2048 µg/mL) (20,000x magnification)
C = The arrow points to *S. aureus* cells that transform into a less spherical shape, and the walls are contracted after exposure to the n-hexane fraction with 8x MIC (1024 µg/mL) (20,000x magnification)
D = The arrow points to *S. aureus* cells that experience change in shape (no longer spherical) and size (smaller) after exposure to the ethyl acetate fraction with 8x MIC (512 1024 µg/mL) (20,000x magnification)

At 20,000x magnification, *S. aureus* cells experienced a morphological change after exposure to the ethanol extract of *A. auricula*. The cell walls were damaged, dented, and shaped into a non-spherical cell (Figure 3B)—compared to Figure 3A where the cells appear to have a smooth surface. The application of the n-hexane fraction contracted the morphology of the cells (Figure 3C), while exposure to the ethyl acetate fraction reduced the size of the cells (Figure 3D). The morphological changes of the *S. aureus* cells are assumed to be the results of the activity of the secondary metabolites contained in the *A. auricula* extracts and fractions.

The Scanning Electron Microscopy (SEM) showed that the bacteria exposed to the *A. auricula* extract with a concentration of 2048 µg/mL (8 x MIC) changed physically—i.e., the cell surface was not entirely round, and there was damage to the cell walls. Change in shape is thought to be the result of alkaloids and phenolic compounds that can alter the characteristics of the cell walls. Meanwhile, the damaged cell walls are believed to be the result of steroids and triterpenoids, which can damage liposomes and cytoplasmic membranes. The bacteria exposed to the n-hexane fraction with a concentration of 1024 µg/mL (8 x MIC) also experienced a change in shape (Figure 3C), i.e., the cells became perfectly round than the normal *S. Aureus* cell whose walls suffered shrinkage. The shrinkage of the cell walls is caused by the lysis of the components of the cell wall. Cell lysis is assumed to be the cause of alkaloids and steroids, which are related to changes in the characteristics of cell walls and

liposome destruction. As a result, the cells become smaller and shrink. Meanwhile, in bacteria exposed to the ethyl acetate fraction at a concentration of 512 µg/mL (8 x MIC), there was a noticeable change in shape (uneven cells) and size (a portion of *S. aureus* cell became small) (Figure D). Changes in shape and morphology in *S. aureus* cells occur due to the presence of phenolic compounds and steroids. Morphological changes after exposure to the extract, n-hexane fraction, and ethyl acetate fraction are assumed to be the result of a compound acting as an antibacterial agent (Ochoa *et al*., 2013).

CONCLUSION

Based on the tests of the antibacterial activities of the extracts and fractions of the wood ear mushroom (*Auricularia auricula*) against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, and *Pseudomonas aeruginosa* using microdilution method, bioautography, and Scanning Electron Microscopy (SEM), the conclusions are as follows. The extracts and fractions of *A. auricula* exhibit antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, and *Pseudomonas aeruginosa.* The Minimum Inhibitory Concentrations (MICs) of the ethanol extract, n-hexane fraction, ethyl acetate fraction, and methanol-water fraction against *S. aureus* were 256, 128, 64, and 2,048 µg/mL, respectively. The MICs of the ethanol extract, n-hexane fraction, ethyl acetate fraction, and methanol-water fraction against *E. coli* and *B. cereus* were >2,048 µg/mL. The MICs of the ethanol extract, n-hexane fraction, and ethyl acetate fraction against *P. aeruginosa* were >2,048 µg/mL, while the MIC of the methanol-water fraction was 2,048 µg/mL. The Minimum Bactericidal Concentrations of the *A. auricula* extracts and fractions were >2,048 µg/mL. The ethyl acetate fraction has the best inhibitory activity against the bacteria *S. aureus* (MIC= 64 µg/mL). Based on the Rf value (i.e., 0.78), the extract, n-hexane fraction, and ethyl acetate fraction of *A. auricula* can change the *Staphylococcus aureus* cells morphologically, including transformation into irregular shape (not perfectly round), cell shrinkage, and cell damage.

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REFERENCES

Aulia A, Thihana & Mirhanuddin. (2007). Potensi ekstrak kayu ulin (*Eusideroxylon zwageri* T et B) dalam menghambat pertumbuhan bakteri *Staphylococcus aureus* secara in vitro. Bioscientiae. 4(1):37-42.

CLSI. (2012). Methods for Dilution Antimicrobial Susceptibility Tests Bacteria That Grow Aerobically ; Approved StandardNinth Edition. Clinical and Laboratory Standards Institute, 32(2): 13-16.

Depkes RI. (2008). *Farmakope Herbal Indonesia*. Jakarta: Departemen Kesehatan Republik Indonesia.

Efendi, Y.N. & Hertiani, T., (2013). Antimicrobial Potency Of Ant-Plant Extract (*Myrmecodia tuberosa* JACK) Against *Candida albicans, Escherichia coli,* And *Staphylococcus aureus*. *Traditional Medicine Journal*, 18(1)(1410-5918) : 53-58.

Hafidh RR, Abdulamir AS, Vern LS, Abu Bakar F, Abas F, Jahanshiri F, Sekawi Z. (2012). Inhibition of Growth of Highly Resistant Bacterial and Fungal Pathogens by a Natural Product. *The Open Microbiology Journal*, 5: 96-106.

Holetz, F.B, Pessini, G.L, Sanches, N.R, Cortez, D.A, Nakamura, C.V., and Filho, B.P. (2002). Screening of Some Plants Used in the Brazillian Folk Medicine for the Treatment of Infectious Diseases, Mem Inst Oswaldo Cruz, Rio de Janeiro, 97.

Kosworo. (2012). Aktivitas Antibakteri Fraksi Polar Ekstrak Etanol Daun Sirsak (*Annona muricata L*) Terhadap *Klebsiella pneumonia* Dan *Staphylococcus epidermidis*. Bachelor Thesis, Fakultas Farmasi: Universitas Muhammadiyah Surakarta, Surakarta.

Kusumaningtyas, E., Astuti, E. & Darmono, (2008). Sensitivitas Metode Bioautografi Kontak dan Agar Overlay dalam Penentuan Senyawa Antikapang. *Jurnal Ilmu Kefarmasian Indonesia*, 6(2).

NCCLS, (2009). Methods for Antimicrobial Susceptibility Test For Bacteria That Grow Aerobically.

Nwachukwu, E., & Uzoeto, H.O. (2010): Antimicrobial Activity of Some Local Mushrooms on Pathogenic Isolates. *Journal of Medicinal Plants Research*, 4(23), 2460-2465.

Ochoa A P, J. M. Moran, Z.G. Giro, A.H. Rodriguez, R.J. Mujawimana, K.T. Gonzales, S.S. Frometa, (2013): *In vitro* antimicrobial activity of total extracts *of* the leaves of *Petiveria alliaceae* L. (Anamu).

Kementrian Kesehatan RI, (2013). Riset Kesehatan Dasar (RISKESDAS). *Badan Penelitian Dan Pengembangan Kesehatan Kementrian Kesehatan Republik Indonesia*.

Shihabudeen., M.S & Priscilla., H. (2010). Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants. International Journal of Pharma Sciences and Research (IJPSR). 1(10).pp. 430-434.

Udu-lbiam, O.E., Ogbu, O., Nworie, O., lbiam, U.A., Agah, M.V., Nnachi, A.U., Ogbu, K.l., & Chukwu, O.S. (2014): Antimicrobial Activities Of Some selected Edible Mushrooms And Species Against Clinical Isolates. *International journal of scientific & technology research,* 3(5), 251-255.

Wasser SP, & Weis AL.(1999). Therapeutic effect of substances occurring in higher Basidiomycetes mushrooms: a modern perspective. Crit Rev Immunol. 19(1):65-96.

Zakiyah., K.M, Winarsih, S.& Soemardini, (2013). Efektivitas Ekstrak Etanol Bunga Mawar (*Rosa damascena* Mill) Sebagai Antimikroba Terhadap bakteri S*almonella thypi* Secara In-vitro. *Jurnal Penelitian*. Fakultas kedokteran, Universitas Brawijaya.