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Antimicrobial activity of Sappan wood extract (*Caesalpinia sappan* L.) against *Streptococcus pneumoniae*

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Abstract---*Streptococcus pneumoniae* is a normal flora in the upper respiratory tract but can cause Acute Respiratory Tract Infection (ARI), peritonitis, sepsis and meningitis. The widespread use of inappropriate antibiotics to treat *Streptococcus pneumoniae* infection causes antibiotic resistance. Meanwhile, *Streptococcus pneumoniae* requires iron in its growth, namely as a cofactor for the ribonucleotide reductase enzyme, which plays a vital role in DNA synthesis. This study aimed to determine the antimicrobial activity of sappan wood extract (SWE) against *Streptococcus pneumoniae* and the role of SWE as iron chelators on antimicrobial activity. The study was conducted by establishing a minimum inhibitory concentration (MIC) and a minimum bactericidal concentration (MBC) against *Streptococcus pneumoniae* and is evaluated using *Total Plate Count* (TPC), the diameter of the inhibitory zone and the measurement of iron levels using *Atomic Absorption Spectrophotometer*. The results showed that the MIC values for *Streptococcus pneumoniae* 10^3 and 10^5 CFU/mL were 32 ppm and 64 ppm, respectively, with the MIC values of 64 ppm and 128 ppm, respectively. SWE at concentrations of 64 ppm and 128 ppm inhibited 100% of bacterial growth 10^3 and 10^5 CFU/mL, respectively, while at concentrations of 32 ppm and 64 ppm inhibited 99.99% and 99.99% growth, respectively, at a bacterial cell density of 10^3 and 10^5 CFU/mL. Sappan wood extract produces a inhibition zone diameter against *Streptococcus pneumoniae* with density cells of 10^8 CFU/mL was identified at a concentration of 512 ppm with a mean \pm SD of 15.43 ± 0.49 mm. There is a reduction in iron levels in

the medium Mueller Hinton broth (MHB) with the addition of iron dextran after the addition of bacteria and EKS. Sappan wood extract can be an alternative antimicrobial because it has antimicrobial activity and is able to bind iron needed by pathogenic bacteria.

Keywords---Iron chelator, *Caesalpinia sappan L*, Minimal Inhibitory Concentration, Minimal Bactericidal Concentration, Streptococcus pneumonia.

Introduction

Streptococcus pneumonia is a normal flora in the upper respiratory tract of humans. It can cause infection when the body's immune system is weak, such as cilia damage that interferes with mucociliary clearance. Streptococcus pneumoniae colonizes uncontrollably, causing clinical abnormalities such as Acute Respiratory Tract Infection (ARI) up to pneumonia.^{1,2} Streptococcus pneumonia is the most common cause of pneumonia in children under five years globally, and 3% of cases are in Indonesia.^{3,4} Streptococcus pneumoniae infection can be treated by giving antibiotics, but inappropriate use of antibiotics can cause resistance.^{5,6} Availability of antibiotics, easy access to various types of antibiotics, weak government regulations, and lack of education in Indonesia are the causes of the increasing incidence of antibiotic resistance.⁶ Changes in penicillin-binding proteins (PBP) are the leading cause of the resistance mechanism in Streptococcus pneumoniae to penicillin class antibiotics. (Hakenbeck R: 2017)

Research conducted by Su et al. showed the presence of Streptococcus pneumonia that is resistant to penicillin and ceftriaxone. Data from WHO shows that 80% of the world's population uses herbal medicine as a primary source to meet health services. Ten percent of all medicinal plants worldwide are in Indonesia. (Su L;2009) Several studies have been carried out that are relevant to the research that the researcher is doing, namely: "Optimization of Caesalpinia sappan L. heartwood extraction procedure to obtain the highest content of brazilin and greatest antibacterial activity" by Sukanya Settharaksa, who said that high temperature provided high total extract yield as well as brazilin content. In contrast, extraction time had little effect on product or brazilin content. Extraction time had a positive effect, while extraction temperature had little effect on the clear zone against *S. aureus*. The largest clear area against *S. epidermidis* was achieved at low extraction temperature and long extraction time. Conversely, short extraction time and high extraction temperature provided the largest clear zone against *P. acnes*. The optimal conditions providing the highest brazilin content were an extraction temperature and extraction time of 95 C and 30 min, respectively. The exact optimal conditions also offered the most excellent antibacterial activity against the three bacteria. Modeled optimal conditions were validated by conducting extraction using these values. The yield and antibacterial activity of the resulting extract demonstrated that the model had a low percentage error. (Sukanya. S; 2019).

One of the medicinal plants used as herbal medicine by the Indonesian people is sappan wood (*Caesalpinia sappan* L.), widely grown in West Sumatra, Yogyakarta, North Sulawesi, and Central Java and Indonesian people use it as a health drink.15-16. In addition, sappan wood is commonly used to relieve pain due to circulation disorders, diarrhea conditions, edema, and antiseptic. (Nirmal N; 2015).

Several previous studies have proven that chopped wood extract can inhibit the growth of bacteria and fungi such as *Aspergillus niger*, *Candida albicans*, *Salmonella typhi*, *Streptococcus faecalis*, *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aerogenosa*, and *Staphylococcus aureus*. Sappan wood contains bioactive alkaloids, brazilin flavonoids, polyphenols, terpenoids, and tannins which are essential components as an inhibitor of bacterial growth. *Streptococcus pneumoniae* requires iron for growth, biofilm formation, and virulence. (Karlina Y; 2016)

Iron is a cofactor of the enzyme ribonucleotide reductase that plays a role in the DNA synthesis of *Streptococcus pneumoniae*, converting ribonucleotides into deoxyribonucleotides and converting some of the deoxyuridines into thymidine. The antimicrobial ability possessed by Sappan wood extract underlies this study's reason. This research can be the basis for developing antimicrobial agents of herbal origin for further empirical therapy as antimicrobials through iron binding to *Streptococcus pneumoniae*.. (Srinivasan R;2012)

Research Methods

Antimicrobial testing was carried out on *Streptococcus pneumoniae* ATCC 49619 obtained from the Microbiology Laboratory, Advanced Biomedical Laboratory, Faculty of Medicine, Padjadjaran University. Sappan Wood is obtained from Wanagama forest, Yogyakarta, Indonesia which is then extracted to obtain sappan wood extract. The research was conducted experimentally and in vitro at the Microbiology Laboratory, Advanced Biomedical Laboratory, Faculty of Medicine, Padjadjaran University and regional health laboratories. The method used to test the antimicrobial activity of the Sappan wood extract was broth macrodilution, agar well diffusion, and AAS was used to measure iron concentration.

Extraction and Fractionation of Secang

Extraction and fractionation methods are adapted from Safitri et al. Chopped wood is dried in an open room without exposure to direct sunlight. After drying, then blended to produce a fine powder. A sappan wood powder weighed as much as 1 kg and was placed in the Buchner funnel, then macerated using 15 L of ethanol solvent for 24 hours and repeated three times. The maceration results are filtered using Whatman Filter Paper No. 2 and then concentrated on getting dry extract using an evaporator at a temperature of 60 °C. Non-polar components such as oil are removed by liquid extraction using 500 ml of n-hexane solvent. Ethanol extract is further evaporated until it is powdered.

Data Collection

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of SWE against *Streptococcus pneumoniae* using Total Plate Count (TPC) parameters, the diameter of inhibition zone using agar diffusion method, and measurement of iron using Atomic Absorption Spectrophotometer.

Minimum Inhibition Concentration Test (MIC) and Minimum Bactericidal Concentration (MBC)

Determination of minimal inhibitory concentration (MIC) of sappan wood extract is carried out by the macrodilution method. Sappan wood extract at a concentration of 1024 ppm is dissolved in dimethylsulfoxide (DMSO). The stock solution is diluted at Mueller Hinton Broth (MHB) with a ratio of 1:1 between a sappan wood extract solution and MHB to obtain a concentration of 512 ppm and diluted again until it obtains different concentrations (512, 256, 128, 64, 32, 16, 8 ppm).

Then the bacterial suspension was diluted to obtain a dilution of 10^{-3} and 10^{-5} . As much as 10% of the final volume was put into a tube containing each different concentration of sappan wood extract. The bacterial suspension was adjusted to the standard, namely McFarland 0.5, the synoculum cell density reached 108 CFU/mL, the inoculum was then diluted to 10^{-3} and 10^{-5} (10^5 and 10^3 CFU/mL). The minimum inhibitory concentration (MIC) was determined by determining the sappan wood extract at the smallest concentration capable of inhibiting the growth of streptococci after incubation for 24 hours.²⁴ Referring to the Manual of Clinical Microbiology, the determination of MIC was carried out by calculating the percentage inhibition of bacterial growth $\geq 80\%$.²⁵

In addition, a control group was also prepared as a comparison consisting of positive control (MHB + bacterial suspension), negative control (MHB), and standard antibiotic control (MHB + bacterial suspension + each standard antibiotic dilution method sensitivity test, namely ampicillin 0.25 ppm and ceftriaxone one ppm.²⁴ All samples are incubated at a temperature of 33-37°C for 20-24 hours in an incubator of 5% CO₂.

Total Plate Count (TPC)

TPC was used to determine the reduction in the number of bacterial colonies in the determination of the minimum bactericidal concentration (MBC). After all the macrodiluted samples were incubated for 20-24 hours, the samples along with positive, negative controls and antibiotics were inoculated on blood agar. Then the blood agar is put into an incubator containing 5% CO₂ at a temperature of 33-37°C for 20-24 hours.

Diffusion agar Wells

Diffusion agar was used to measure the inhibition zone of sappan wood extract in various concentrations against *Streptococcus pneumoniae*. A McFarland standardized inoculum 0.5 (108 CFU/mL) was inoculated on the blood agar.²⁵ Then, the blood agar was perforated to make a well 6 mm in diameter. A total of 100 L of 7 different sappan wood extract solution concentrations was inserted into the well. Antibiotic control used standard discs for sensitivity testing, namely ampicillin 10 g (equivalent to 100 ppm) and ceftriaxone 30 g (equal to 300 ppm).²⁴ All samples were repeated three times (triple). The blood agar was incubated at 33-37°C for 20-24 hours in a 5% CO₂ incubator. Response of bacterial growth inhibition by EKS from Lim et al. refers to the classification of potential response (++++) with a diameter zone >30 mm; strong response (+++) with a zone diameter of 21-30 mm; moderate response (++) with a diameter zone of 16-20 mm and a weak response (+) with a diameter zone of 10-15 mm.

(Safitri, R;2017)

Atomic Absorption Spectrophotometer (AAS)

Iron concentration reduction measurements are carried out using AAS, and these measurements aim to see if there is a growing dependence of *Streptococcus pneumoniae* on iron. There are four groups to be measured, namely Mueller Hinton Broth (MHB)+iron dextran (iron), MHB+*Streptococcus pneumoniae*+iron dextran, MHB + a solution of a wood extract with concentrations of KHM, and KBM +iron dextran, MHB+*Streptococcus pneumoniae*+a solution of a wood extract with concentrations of KHM and KBM. The four groups were subdivided in a 5% CO₂ incubator at 33-37°C for 20-24 hours; then, their free iron concentrations were measured using an Atomic Absorption Spectrophotometer (AAS).

Data Analysis

Quantitative data results in the form of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), percentage reduction of iron, and sappan wood extract concentration that affects the growth of *Streptococcus pneumoniae* descriptively.

Theoretical Concepts

Streptococcus pneumoniae is a normal flora that resides in the upper respiratory tract of humans.¹ These bacteria enter the body through secretion contact and droplet transmission through the air when sneezing or coughing from individuals, colonizing, and dynamically competitively interacting with the host. (WHO;2014) The host's innate and adaptive immune responses play an essential role in maintaining a balance of interactions against *Streptococcus pneumoniae*. Conditions such as cilia damage will interfere with mucociliary clearance, so *Streptococcus pneumoniae* will colonize more uncontrolled, causing clinical abnormalities such as Acute Respiratory Tract Infection (ISPA) to pneumonia. (Henriques-Normark;2013) The most common diseases caused by *Streptococcus pneumoniae* infection are ISPA, namely sinusitis, and otitis media. (Kandakai-

Olukemi Y;2009) In addition to ISPA, Streptococcus pneumonia can cause bronchitis, bacteremia, meningitis, and peritonitis.1 Streptococcus pneumonia is the most common cause of pneumonia in children under five years of age in the world, and 3% of cases are in Indonesia. (O'Brien K;2009)

Lung infections are responsible for 16% of deaths in children under five years of age globally and recorded 920,136 deaths in 2015. (Oyebode O;2016) Acute infections of the respiratory tract are treated by antibiotic administration. Still, improper use of antibiotics can lead to resistance. In addition, antibiotic resistance can occur naturally. Changes in penicillin-binding proteins (PBP) are the leading cause of the resistance mechanism in Streptococcus pneumonia to penicillin-class antibiotics. Research conducted by Linhui et al. showed the presence of Streptococcus pneumonia that is resistant to penicillin and seftriacson. (Bortoni M;2009).

Other studies also showed that Streptococcus pneumoniae is resistant to gentamicin. Therefore, research is needed to create new antimicrobial agents that are not yet resistant and can inhibit and kill Streptococcus pneumoniae. According to the World Health Organization (WHO) shows that 80% of the world's population uses herbal medicine as the primary source to meet health services. One of the medicinal plants used as herbal medicine by the people of Indonesia is secang (Caesalpinia sappan L.). (WHO; 2004)

Sappan wood is widely grown in Indonesia, especially in West Sumatra, Yogyakarta, North Sulawesi, and Central Java. Sappan wood is used as a traditional treatment for dengue and microbial infections. Several previous studies have proven that chopped wood extract can inhibit the growth of bacteria and fungi. Sappan wood contains bioactive alkaloids, Brazilian flavonoids, polyphenols, terpenoids, and tannins which are important components as inhibitors of bacterial growth. Sappan wood contains bioactive compounds such as brazilin, 1', 4'-dihydrospiro [benzofuran-3 (2H), 3' - [3H -2] benzopyran] -1', 6', 6', 7'-tetrols, and 3 - [[4,5-dihydroxy-2 (hydroxymethyl) phenyl] methyl] -2-3-dihydro-3,6-benzofurandiols which is able to inhibit xanthine oxidase activity, capture superoxide anion radical, free hydroxyl radicals, and is indicated as a source of iron chelation. (Safitri R ;2002) Brazilin has antioxidant, antibacterial, antifungal, anti-inflammatory, anti-photoaging, vasorelaxant, hepatoprotective, and anti-acne activities. Flavonoids have an antibacterial role. Ethanol extracts and wood methanol play an important role in inhibiting the growth of bacteria and fungi. In addition, in vivo tests showed that chopped wood extract could be used as an iron class agent as evidenced by the ability to reduce ferritin, hepatic iron level, serum iron level, transferrin saturation, increase transferrin and TIBC levels in mice in excess iron conditions. (Safitri, R;2017)

Streptococcus pneumonia

Streptococcus pneumonia is a gram-positive bacteria, catalase-negative, inulin positive, nonmotile, facultatively anaerobic, sensitive to optochin, positive quellung reaction, non-spore-forming, mesophilic, and fastidious found 4-50% as normal flora in the human nasopharynx. In the unpaired state, Streptococcus pneumonia has a diameter of 0.5-1.25 μ m. Streptococcus pneumonia has more

than 90 serotypes distinguished by their C-polysaccharide capsule. The *Streptococcus pneumoniae* polysaccharide capsule is the main virulence factor that can prevent phagocytosis and C3b activation. Pneumolysin can induce cytokines and disrupt cilia in the respiratory tract. Peptidoglycan will induce inflammation. The cell wall consists of teichoic acid, LPA, and phosphocholine attached to peptidoglycan and extends to the capsule. Choline binding proteins (CBPs) attach to the cell walls of choline pneumococci and carbohydrates that appear on the surface of epithelial cells. (Carroll K; 2016)

The role of LPXTG protein as virulence factors is hyaluronidase, neuraminidase, and serine protease PrtA. Hyaluronidase destroys hyaluronic acid components in mammals' connective tissue and extracellular matrix. (Goering R; 2012) Hyaluronidase allows inflammation of the lungs by interacting with proinflammatory cytokines and chemokines. Neuraminidase breaks down N-acetylneuraminic acid from glycolipids, lipoproteins, and oligosaccharides on the cell surface, causing sialic acid loss. (Ray C; 2014) Interactions between *Streptococcus pneumoniae*-secreted products, the bacterial components attached to the alveolus epithelium, and innate immune defenses will induce pneumonia. Pneumolysin will perforate the epithelium of the alveolus, while hydrogen peroxide will interfere with the epithelium of the alveolus and cause the edema fluid to gather in the alveolus chamber. The multiplication of *Streptococcus pneumoniae* in alveolus produces pneumococcal pathogen-associated molecular patterns (PAMPs) that will be recognized by innate immune so that it will initiate the deployment of neutrophils to the site of infection and produce hemorrhagic debris. *Streptococcus pneumoniae* is also known to some toll-like receptors (TLRs), such as TLR2 (pneumococcal lipoteichoic acid [LTA] as a ligand), TLR4 (recognizing pneumolysin), and TLR9 (interacting with bacterial DNA). Macrophage receptor with collagenous structure (MARCO) expressed by macrophage alveolus contributes to the innate immune response in the lungs, muramyl dipeptide, which is a component of pneumococcal peptidoglycan (MDP-PG) recognized by the nucleotide-binding oligomerization domain (NOD-2) then activates the host defense and inflammation.

Bacterial culture is carried out using blood agar which will later produce partial hemolysis or hemolysis. Its growth can be increased by 5-10% CO₂. Incubation was conducted at 37°C, forming small spherical, initially dome-shaped colonies, which later developed into central depression and high margins; the colonies were shiny due to the polysaccharide capsule.¹ Until now, the treatment of bacterial infection with *Streptococcus pneumoniae* uses the antibiotics ampicillin, gentamicin, and chloramphenicol as the first line and ceftriaxone as the second line.³² However, *Streptococcus pneumoniae* can produce β lactamase, which plays a role in resistance to ampicillin.³³ Further research also showed that *Streptococcus pneumoniae* could experience drug resistance to erythromycin at 45%, clindamycin at 45%, and chloramphenicol at 0.56%. (Karcic E; 2015)

Antibiotic sensitivity tests for *Streptococcus pneumoniae* may use disc diffusion and broth microdynamict. *S. pneumoniae* uses iron for virulence factor ability, grows, and maintains its survival; blocking or disrupting iron acquisition systems can alter iron homeostasis and suppress bacterial growth. *Streptococcus pneumoniae* also requires iron for viability and the expression of two membrane

proteins that bind to hemoglobin and haem. In addition to iron playing a role in the formation of DNA, biofilms and plays a role in cell metabolism, research conducted by Trappetti et al. shows that Fe³⁺ supplementation increases the formation of biofilms and iron class will worsen the process of biofilm formation. (Romero-Espejel; 2013)

Results and Discussions

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Sappan Wood Extract (*Caesalpinia sappan L.*) against *Streptococcus pneumoniae*.

MIC and MBC are determined through inhibiting and killing concentrations and reductions in bacterial growth. A reduction (%) in the number of bacteria after treatment with antimicrobial agents is presented in the following table.

Table 1
MIC and MBC of Sappan Wood Extract (*Caesalpinia sappan L.*) against *Streptococcus pneumoniae*

Antibiotics and EX concentrations	Growth in diluted bacteria 10 ⁵ CFU/mL			Growth in bacterial dilution 10 ³ CFU/mL			Reduction of bacteria in dilution 10 ⁵ CFU/mL	Reduction of bacteria in dilution 10 ³ CFU/mL
	S	D	T	S	D	T		
Negative Control	-	-	-	-	-	-	0%	0%
Positive Control	+	+	+	+	+	+	0%	0%
Ampisilin 0,25 ppm	+	+	+	+	+	+	0%	0%
Seftriakson 1 ppm	+	+	+	-	-	-		100%
Sappan Wood Extract (ppm)								
8	+	+	+	+	+	+	0%	0%
16	+	+	+	+	+	+	97,52174%	80,99379%
32	+	+	+	+	+	+	99,99904%	99,99999%
64	+	+	+	-	-	-	99,99966%	100%
128	-	-	-	-	-	-	100%	100%
256	-	-	-	-	-	-	100%	100%
512	-	-	-	-	-	-	100%	100%

Information S: Simplo, D: Duplo, T: Triplo, Kontrol Negatif: *Mueller Hinton Broth* (MHB), Positive Control: MHB + *Streptococcus pneumoniae*, MIC, MBC.

Table 1. Shows streptococcus pneumoniae growth in the inoculum dilution 10⁻³ and 10⁻⁵ (10⁵ and 10³ CFU/mL) after adding chopped wood extract, positive, negative control, and antibiotics. On dilution 10⁵ CFU/mL After adding wood extract, a concentration of 64 ppm is still visible bacterial growth, but bacterial growth is no longer visible at a concentration of 128 ppm. While on 10³, The concentration of 32 ppm is still visible bacterial growth, and bacterial growth is

no longer visible at a concentration of 64 ppm. While the control of ampicillin antibiotics in *Streptococcus pneumoniae* 105 dan 103 CFU/mL showed positive results. In addition, control of seftriakson antibiotics in bacteria 105 CFU/mL Also shows positive results.

A percentage reduction in the number of *Streptococcus pneumoniae* colonies in dilution of inoculum 105 and 103 CFU/mL is carried out to confirm the number of colonies. The results showed a reduction percentage of *Streptococcus pneumoniae* of 105 CFU /mL at 64 ppm (MIC), which is 99.9%, at 128 ppm (MBC), which is 100%, while *Streptococcus pneumoniae* at 103 CFU / mL at 32 ppm (MIC) is 99.9% and at 64 ppm (MBC) which is 100%. MIC and MBC of SWE in 10^5 CFU/mL bacteria were higher than 103 CFU/mL bacteria because at 10^5 CFU/mL the number of bacteria was higher so that the SWE concentration needed to inhibit and kill *Streptococcus pneumoniae* was also higher than 103 CFU/mL.

Diameter of The Inhibitory Zone of Sappan Wood Extract (*Caesalpinia sappan* L.) in *Streptococcus pneumoniae*

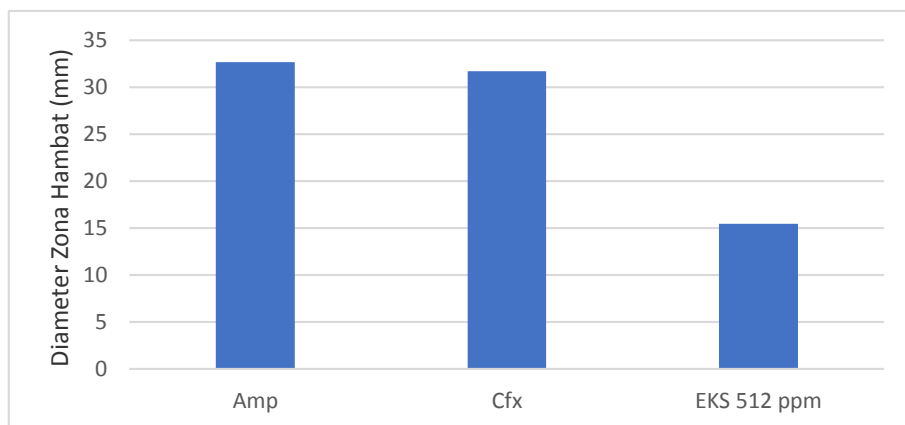


Figure 1. Diameter of the inhibitory zone of wood extract as fast as against *Streptococcus pneumoniae* at a cell density of 108 CFU/mL

Description: Amp:

Ampicillin 10 µg (equivalent to 100 ppm), Cfx: Seftriakson 30 µg (equivalent to 300 ppm), SWE : Sappan Wood Extract

Figure 1. demonstrated the ability of chopped wood extract as an antimicrobial against McFarland-standardized *Streptococcus pneumoniae* 0.5 (equivalent to 108 CFU/mL). The diameter of the inhibitory zone produced by wood extracts against *Streptococcus pneumoniae* is seen at a concentration of 512 ppm with an average diameter of 15.43 ± 0.49 mm, which falls into the category of weak response. Meanwhile, the antibiotic control group results showed an average diameter of the inhibitory zone more significant than 512 ppm sappan wood extract, which was 32.63 ± 0.34 mm for ampicillin and 31.69 ± 0.56 mm for ceftriaxone which fell into the category of potent response based on categorization due to >30 mm.

Activity of Sappan Wood Extract as an Iron Chelating Agent Inhibiting the Growth of *Streptococcus pneumoniae*

Measurement of iron content reduction using an Atomic Absorption Spectrophotometer (AAS) was carried out to determine the effect of iron chelation of sappan wood extract that inhibited the growth of *Streptococcus pneumoniae* seen from the level of iron reduction tested in several groups and carried out with three repetitions (triple). The decrease in iron content can be seen in Figures 2, 3, and 4.

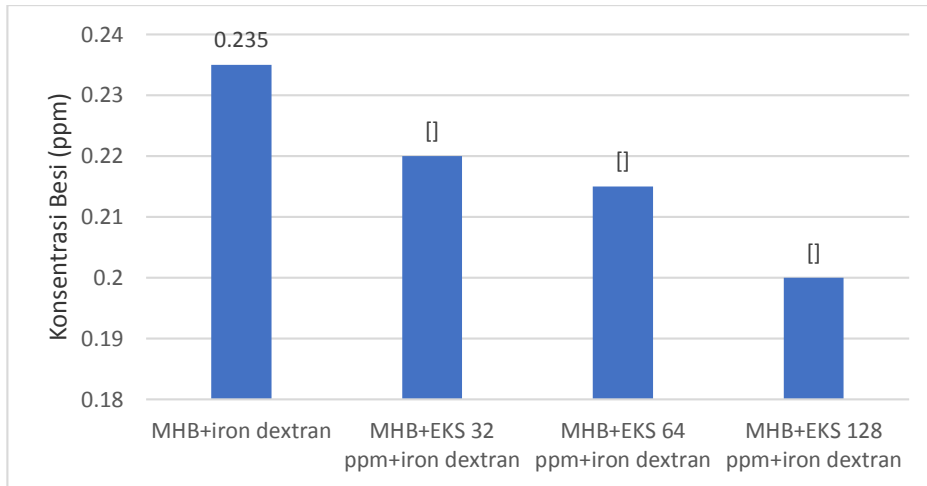


Figure 2. Iron Concentration (Fe) in MHB with the addition of iron dextran and addition of Sappan Wood Extract

Description: MHB: Mueller Hinton Broth, SWE Sappan Wood Extract

The results showed that MHB (Figures 2.3 and 4) added iron dextran and inoculated *Streptococcus pneumoniae* in dilution of inoculum 10⁻³ and 10⁻⁵ (CFU/mL). Groups without bacterial inoculation showed differences in iron concentrations. The iron concentration of the MHB+iron dextran+bacterial group is lower than the MHB+iron dextran group without bacteria. This shows that *Streptococcus pneumoniae* at dilution 10³ CFU/mL uses 10.63% iron from MHB+iron dextran, while *Streptococcus pneumoniae* 10⁵ CFU/mL uses 23.4% iron from MHB+iron dextran.

The iron chelation effect of SWE showed that SWE could chelate iron compared to the control MHB + iron dextran without the addition of SWE. There was a reduction in free iron content in MHB+iron dextran by SWE 32 ppm by 6.38%, SWE 64 ppm by 8.5%, and SWE 128 ppm by 14.89%. The concentration of free iron in MHB+iron dextran was higher than that of MHB+iron dextran added with SWE. The ability of SWE to chelate iron was clarified by providing a greater concentration of SWE; the free iron content in MHB+iron dextran is also reduced.

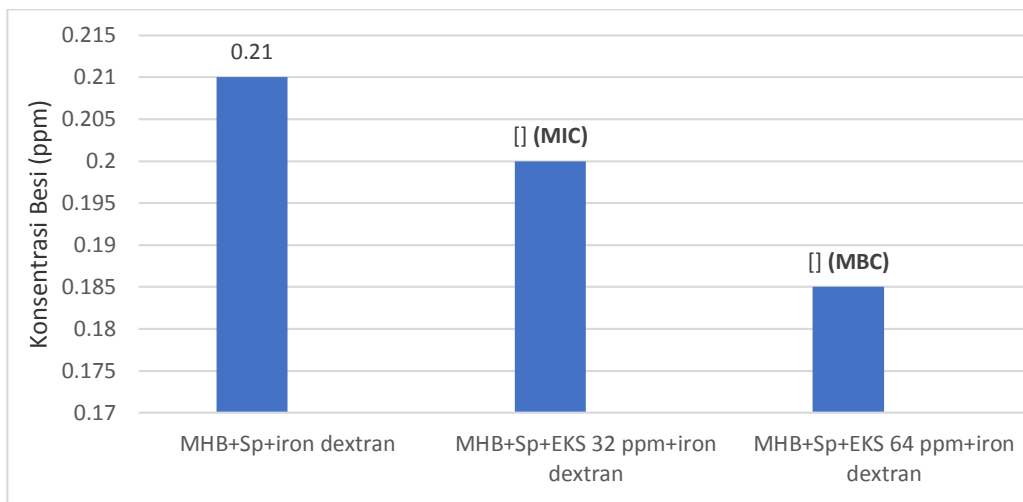


Figure 3. Concentration of Iron (Fe) in MHB+bacteria 10^3 CFU/mL+iron dextran that has been incubated

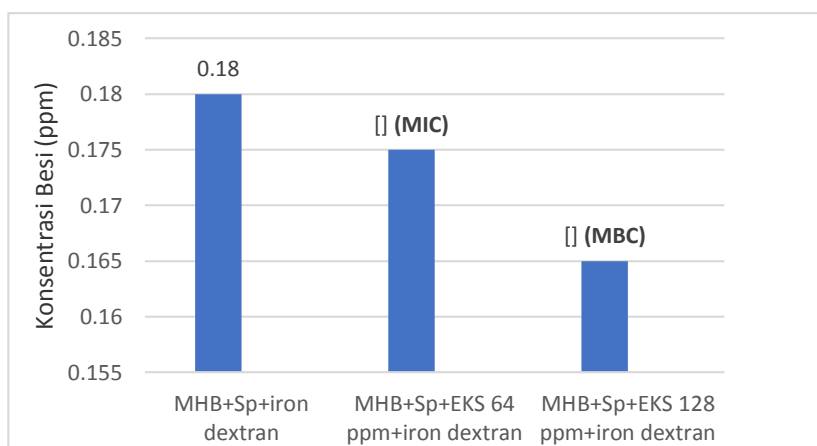


Figure 4. Iron Concentration (Fe) in MHB + iron dextran coupled with bacteria with 105 CFU /mL dilution.

Description: MHB: Mueller Hinton Broth, Sp: Streptococcus pneumoniae, SWE : Sappan Wood Extract

Sappan wood extracts at concentrations of 32 ppm and 64 ppm (Figure 3.) can chelate iron by 4.76% and 11.9% of the MHB +iron dextran medium, respectively, with the addition of bacteria with a cell density of 10^3 CFU/mL. Figure 4 shows that SWE 64 ppm and 128 ppm can chelate iron by 2.7% and 8.3% of the MHB++iron dextran medium, respectively, in bacteria at 105 CFU/mL.

Discussion

Streptococcus pneumonia in dilution 105 and 103 CFU/mL has different KHM and KBM, as shown in Table 1. 105 CFU/mL bacteria have KHM and KBM than

bacteria 103 because they contain a more significant number of bacteria. At the same time, higher concentrations of EKS are needed to inhibit and kill more bacteria. There have been several studies on the antimicrobial activity of EKS against some bacteria. Alkaloids and tannins in EKS have inhibited the cell wall so that bacterial growth can be inhibited.^{13,36} In addition, other studies suggest that brazilin, as the main bioactive in chopped wood, can inhibit protein and DNA synthesis. The percentage reduction in the number of bacteria confirms that EKS at specific concentrations has different antimicrobial activity. The greater the concentration of EKS, the more significant the reduction in the number of bacteria.

In this study, the antimicrobial activity of SWE was compared with standard ampicillin and ceftriaxone antibiotics to test for *Streptococcus pneumoniae* ATCC 49619 sensitivity. It was also an empirical therapy for streptococcus pneumonia clinical infection. Meanwhile, the antimicrobial activity of ampicillin 10 g (100 ppm) and ceftriaxone 30 g (300 ppm) has a potent inhibitory zone compared to SWE, which at 512 ppm has a weak inhibitory zone. The result proves that at low antibiotic concentrations, it can kill more *Streptococcus pneumoniae* than SWE by interfering with bacterial wall synthesis. Standard antibiotics have potent antimicrobial activity against *Streptococcus pneumoniae*, but unfortunately, antibiotics have harmful side effects such as severe allergic reactions and developing resistance (Shalviri G;2013)

Figure 2 shows the ability of SWE to chelate iron, this is evidenced by comparing MHB+iron dextran with the MHB+SWE group at various concentrations of +iron dextran. MHB+iron dextran contains more free iron than MHB+SWE at various concentrations+iron dextran. Figures 3 and 4 show that 32 ppm SWE has lower iron chelating ability than 64 ppm SWE, and 64 ppm SWE has lower iron chelation ability than 128 ppm SWE because it contains lower iron chelating substances. Meanwhile, the iron concentration in MHB+bacteria+SWE+iron dextran was lower than in MHB+bacteria+iron dextran without the addition of SWE. The braziline compound in SWE has an indication as a source for iron chelation.²⁹ The antimicrobial effect of SWE is better than other plants because it has more bioactive compounds such as brazilin, phenol, alkaloids and tannins. Brazilin is a phenol derivative that plays a role in iron chelation with a redox mechanism.

The ability of *Streptococcus pneumoniae* to use iron is demonstrated in Figures 3 and 4, at 103 CFU/mL using 10.63% iron and at 105 CFU/mL using 23.4% iron. *Streptococcus pneumoniae* has gene expression for virulence, such as *cps4A*, *zmpA*, and *pavA*, which is high in the presence of iron.²⁰ *Streptococcus pneumoniae* has a *LuxS* gene that mediates iron-dependent biofilm formation and also has an iron transport system that, when disturbed, reduces bacterial iron intake and can suppress growth. bacteria.^{21,30-31} In addition, *Streptococcus pneumoniae* also requires iron as a cofactor for the ribonucleotide reductase enzyme, which plays an important role in DNA synthesis for its growth

The iron level measurements showed the possibility of iron use competition between SWE and *Streptococcus pneumoniae*. Brazilin is a homoisoflavonoid, a form of phenol compound in chopped wood as the main active compound that

plays a role in antimicrobial activity by inhibiting protein and DNA synthesis. SWE has been known to be a safe plant extract, has long been used as safe food and medicinal ingredient, and does not cause acute toxicity in male and female mice with parameters of behavior changes and changes in internal organs. In comparison, antibiotics have serious side effects and are prone to resistance. Therefore, the SWE mechanism that can chelate iron in the extracellular environment certainly has an antibacterial mechanism different from antibiotics that work intracellularly. With this difference in antimicrobial mechanisms, the use of SWE can be developed as a new antimicrobial agent against *Streptococcus pneumoniae*.

Conclusion

The MIC value of *Streptococcus pneumoniae* evidences the ability of sappan wood extract as an antimicrobial at 103 and 105 CFU / mL at 32 ppm and 64 ppm, respectively. In contrast, MBC against *Streptococcus pneumoniae* 103 and 105 is 64 ppm and 128 ppm, respectively. The inhibitory zone diameter appears at a concentration of 512 ppm of sappan wood extract (15.43±0.49 mm). The antimicrobial activity of Sappan wood extract can be related to compounds contained in sappan wood extracts that can chelate iron. This study proves a reduction in the number of bacterial colonies and the concentration of free iron in MHB. The results of this study could be the basis for future research for empirical therapy of diseases caused by *Streptococcus pneumoniae*.

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