

## ANTIBACTERIAL SYNERGY DETECTION OF LIGNIN EXTRACT FROM OIL PALM EMPTY FRUIT BUNCHES (OPEFB) COMBINED WITH AMOXICILLIN AGAINST *Staphylococcus Aureus* USING THE Azdast METHOD

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**Abstract.** Oil palm empty fruit bunches (OPEFB) are waste generated by the palm oil industry and are generally considered an environmental problem due to their large quantity and difficult recyclability. This study's objective was to examine the potential of OPEFB lignin extract combined with amoxicillin in inhibiting the growth of *Staphylococcus aureus* bacteria. The Synergism Test of the OPEFB lignin extract-antibiotic combination was conducted using the AZDAST method (Ameri Ziaei Double Antibiotic Synergism Test). The lignin extraction process was a steam process using a 4% oxalic acid solution under optimal conditions. Then, Calcium ions precipitate 4% oxalate by adding  $\text{Ca}(\text{OH})_2$  to the extract. The Lignin content was determined by UV-Vis spectrophotometry at 280 nm, and then the lignin extract was combined with antibiotics for antibacterial assay. The results showed a lignin content of 1.06 %. The AZDAST test results revealed clear zones from the combination of OPEFB lignin extract and amoxicillin with a diameter of 20.23 mm for the extract containing oxalate and 15.83 mm for the oxalate-free extract. Meanwhile, on average, the clear zone of single amoxicillin was only 14.13 mm. Based on these results, the combination of OPEFB lignin extract with amoxicillin was stronger than amoxicillin alone in inhibiting the growth of *S. aureus*, but it was not significantly different ( $p\text{-value} > 0.05$ ). In conclusion, the potential of OPEFB lignin extract synergistically worked with the antibiotic amoxicillin in inhibiting the growth of *S. aureus*.

**Keywords:** OPEFB; Lignin; Antibacterial Synergy; *Staphylococcus aureus*; AZDAST Method

### 1. Introduction

Oil palm empty fruit bunches (OPEFB) are environmental waste generated after palm oil production. Indonesia, currently the world's largest palm oil producer, can produce 45.12 million tons of palm oil, according to the Central Statistics Agency in 2021 (BPS, 2022). Each ton of palm oil leaves approximately 230 kg (23%) of OPEFB waste. While the utilization of OPEFB waste is commonly limited to organic fertilizer and mushroom cultivation media, the remaining waste is often left untreated or subjected to burning (Dirgantoro & Adawiyah, 2018). Therefore, it is crucial to conduct further exploration to enhance the value of OPEFB. According to Hidayah and Wusko (2020), OPEFB contains lignocellulose, which consists of lignin, cellulose, and hemicellulose.

Lignin plays a role in maintaining plant rigidity and possesses antimicrobial properties (Tribot *et al.*, 2019). Several studies have mentioned the positive effects of lignin in inhibiting

microbial growth. Lignin in the form of nanoparticles has been utilized as an antibacterial agent in food packaging (Morena & Tzanov, 2022). In a study conducted by Yun *et al.* (2021), lignin extracted from Moso Bamboo (*Phyllostachys edulis*) demonstrated the ability to inhibit the growth of *S. aureus* bacteria. The report by Wang *et al.* (2019) states that lignin from Bamboo (*Phyllostachys pubescens*) can act as a natural bacteriostatic agent. Similarly, research reports from Dong *et al.* (2011) stated that the antimicrobial activity of lignin extracted from corn stover residue also showed positive results against *S. aureus* bacteria. Other tests have reported that lignin exhibits strong antimicrobial activity against Gram-positive bacteria (Alzagameem *et al.*, 2019). Lignin belongs to the phenolic group because it is composed of polyphenolic compounds. The major monomers forming the lignin structure are aromatic compounds, such as p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) alcohols. Each of these compounds originates from different precursors, namely p-coumaryl, coniferyl, and sinapyl alcohols (Kai *et al.*, 2018). Previously reported by Wei *et al.* (2022), it was found that lignin precursors derived from coniferyl and sinapyl alcohols exhibit strong antibacterial activity against *S. aureus*.

The extraction of lignin in this research was conducted through delignification using oxalic acid dihydrate, partially referring to the report by Suryadi *et al.* (2020). The extraction was performed at a temperature of 121°C and processed for 90 minutes after reaching the target temperature. Subsequently, the mixture was filtered to obtain a filtrate in the form of a liquid extract. The remaining oxalate in the extract was precipitated using Ca(OH)<sub>2</sub>, producing oxalate-free lignin extract. The obtained lignin compound was quantified using a UV-Vis spectrophotometer to analyze the lignin content of the extraction product. Finally, the antibacterial synergistic test of the extracts with antibiotics against bacteria was conducted. The antibacterial synergistic test of plant extracts combined with antibiotics has been reported by Saquib *et al.* (2021), showing significant antibacterial activity against all tested bacteria. The study further proceeded with the assessment of antibacterial synergy using the Ameri-Ziaei double antibiotic synergism test (AZDAST) method.

The AZDAST method was employed to investigate the potential of lignin extract from OPEFB powder in combination with antibiotics to inhibit the growth of *Staphylococcus aureus* bacteria. The advantage of the AZDAST method lies in its qualitative analysis, yet with a numerical scale that can be interpreted by comparing the diameter of the inhibition zones in millimetres. The results can stand alone without the need for standard tables. The quantitative amount of each test sample can be calculated using a quantitative method (Ziaei-Daroukalei *et al.*, 2016). Till now, there have been no further reports on the antibacterial activity combination of OPEFB lignin extract and antibiotics. Therefore, the objective of this research was to evaluate the

antibacterial activity of the combination of OPEFB lignin extract and amoxicillin against *Staphylococcus aureus* bacteria.

## 2. Methods

### 2.1. Tools and materials

The sample tested in this research is oil palm empty fruit bunches (OPEFB) collected from Pasaman Barat (West Sumatra Province, Indonesia). Some of the equipment used in this research include autoclave (Tomy Kogyo Co. Ltd., Japan), UV-Vis spectrophotometer (Shimadzu, Japan), laminar air flow (ESCO, Singapore), oven (Lab-Line Instruments Inc., Melrose Park–USA), incubator (Mettler, Germany), vortex (Barnstead International, USA) and grinding machine. Meanwhile, some of the materials used in this research include aqua pro-injection (PT. Ikapharmindo Putramas, Jakarta–Indonesia), oxalic acid dihydrate (Merck, Germany), calcium hydroxide (Merck, Germany), sodium lignosulfonate, Mueller Hinton agar (Oxoid, England), nutrient agar (Merck, Germany), *Staphylococcus aureus* ATCC 25923 colony (Laboratory of Pharmaceutical Microbiology and Biotechnology at Faculty of Pharmacy – Universitas Indonesia, Indonesia), sodium chloride 0.9% (PT. Widatra Bhakti, Jakarta – Indonesia), and Amoxicillin (Sigma, Germany).

### 2.2. Research Design

The research design began with collecting OPEFB, which were then dried and ground into powder form. Subsequently, extraction was performed using a steam process at high pressure and temperature, employing a solution of oxalic acid dihydrate. The resulting mixture was then filtered to obtain a liquid extract. The extract contained lignin, which was the product of the extraction process from the OPEFB powder. Further treatment was applied to precipitate the oxalate present in the extract. The subsequent step involved analysis using a UV-Vis spectrophotometer, followed by an antibacterial synergy test against *S. aureus* using the AZDAST method. Finally, the inhibition zone results were analyzed statistically using SPSS 24 software. The research design flow diagram can be seen in [Figure 1](#) below.

### 2.3. OPEFB Powder Preparation

Collection of OPEFB involved cleaning them from impurities using running water until they were thoroughly clean. Subsequently, the OPEFB were sun-dried and air-dried for 3 days. They were then cut into small pieces and finely ground using a milling machine and blender, then dried in an oven at 60°C for 24 hours. Afterwards, they were stored in a sealed container with silica gel pouches.

### 2.4. OPEFB Lignin Extraction and Oxalate Precipitation

Lignin extract from the OPEFB fibers was obtained through a delignification method. The

method used was based on that carried out by [Suryadi et al. \(2020\)](#), with some modifications. This process involved steaming the sample in an autoclave at a temperature of 121°C and a pressure of 1 atm for 90 minutes. Initially, heat-resistant vials with a size of 100 mL were prepared. Then, 3 grams of OPEFB powder were added to the vial and 18 mL of a 4% oxalic acid dihydrate solution (at a ratio of 1:6, w/v). After the steaming process, the solution in the vial was filtered, and the resulting filtrate was collected as the liquid extract.

The obtained extract still required further processing by precipitating the oxalate with an addition of  $\text{Ca}(\text{OH})_2$ . Initially, 235.07 mg of  $\text{Ca}(\text{OH})_2$  was weighed and placed into a 50 mL glass beaker. Next, 10 mL of the extract was added, followed by stirring using a magnetic stirrer at 200 rpm for 2 minutes or until no further precipitation was observed. Finally, the mixture was filtered to collect the filtrate, representing the extract now free from oxalate.

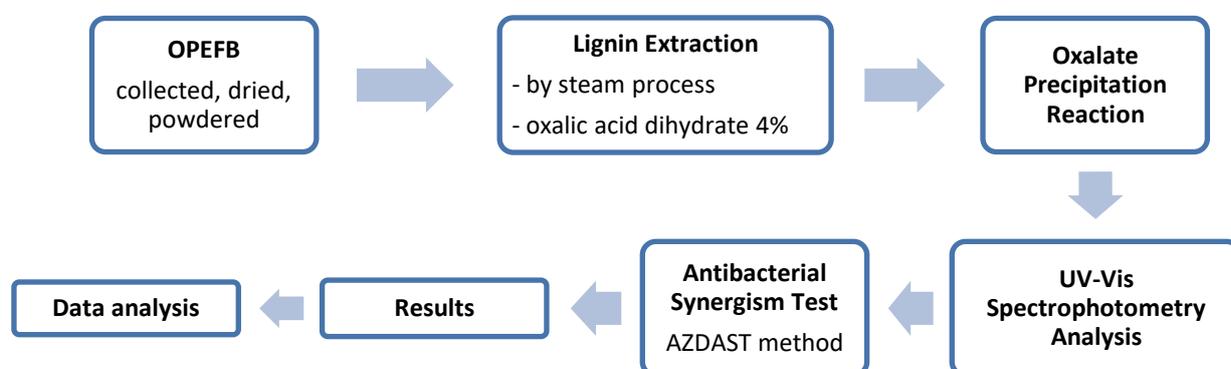


Figure 1. Research design flowchart

## 2.5. Determination of Lignin Content in OPEFB Extract by UV-Vis Spectrophotometry

The procedure for determining lignin content refers to the research of [Risanto et al. \(2014\)](#), modified. The maximum wavelength of lignin in the 200 - 400 nm range was determined. Sodium lignosulfonate was used as the reference standard in the study. The initial step involved creating a calibration curve using a stock solution. 50 mg of sodium lignosulfonate was weighed and diluted with distilled water to a total volume of 100 mL. From the stock solution, several concentrations were prepared, namely 30 ppm, 40 ppm, 50 ppm, 60 ppm, 70 ppm, and 80 ppm. The absorbance was measured at the maximum wavelength based on the obtained results. Subsequently, for the analysis of lignin content in the OPEFB extract, 0.5 mL of the extract was taken and diluted to a final volume of 10 mL with distilled water. The absorbance readings were conducted at the maximum wavelength obtained from the calibration curve of the stock solution. Three replicates were performed for absorbance readings of the samples.

## 2.6. Antibacterial Synergy Test by The AZDAST Method

The method used in this study was based on [Ziaei-Daroukalei et al. \(2016\)](#). Firstly, a medium for disc adhesive (called glue) was prepared using sterilized Mueller Hinton agar (MHA), with a concentration 1.5 times higher than usual. During the disc placement process, the glue was maintained in a liquid state (44 - 48°C). Sterilized petri dishes were prepared, and the discs moistened with the glue were placed on the bottom of the dishes. Once all the discs were placed, liquid MHA medium mixed with a suspension of *S. aureus* bacteria was poured into the dishes until the media level submerged all the discs. The dishes were then allowed to solidify in a closed state. Subsequently, they were incubated at 37°C for 24 hours. Finally, the resulting clear zones were observed measured.

## 2.7. Design Analysis

The clear zones formed in the antibacterial synergy test using the AZDAST method were measured using a millimetre calliper (mm). Data were processed using Microsoft Excel software to obtain the mean values and standard deviations. The determination of the type of interaction based on the AZDAST test results was interpreted using [Table 1](#). Analysis of the significance of inhibition zone data was carried out using non-parametric statistical tests with the Mann-Whitney method, which was processed in SPSS 24 software.

Table 1. Interpretation of combination interaction types on the AZDAST test

AZDAST test combination results	Interpretation
If $AB > A$ & $AB > B$ , and $AB > AA$ & $BB$ / $AB < AA$ & $BB$ / $AB > AA$ although $AB < BB$ / $AB > BB$ although $AB < AA$	Synergistic
If $A$ or $B = 0$ , which is $AB > A$ & $AB > B$ , and $AB > AA$ & $BB$ / $AB < AA$ & $BB$ / $AB > AA$ although $AB < BB$ / $AB > BB$ although $AB < AA$	Potential (enhancement)
If $AB < A$ atau $AB < B$	Antagonistic
If $AB = AA$ and $BB$ , or $AB = AA$ only or $AB = BB$ only	Addictive
If $AB = A$ or $AB = B$	Indistinguishable

([Ziaei-Daroukalei et al., 2016](#))

## 3. Results and Discussion

### 3.1. OPEFB Powder Preparation

The OPEFB washing process aims to remove impurities such as dust and dirt, slightly reducing weight. Subsequently, the OPEFB is dried to reduce its moisture content, leading to a decrease in weight. Further drying using an oven helps eliminate any remaining moisture in the

OPEFB. Finally, during the grinding of the OPEFB into powder form, weight loss may occur due to the grinding and dispersion process. As a result, the weight of the OPEFB decreases from the initial 1.1 kg to 0.32 kg of OPEFB powder after the preparation process (see [Table 2](#) and [Figure 2](#)).

Table 2. OPEFB weight before and after preparation

Parameter	Weight (kg)
Gross weight	1.10
Net weight (after cleaning and drying)	0.32

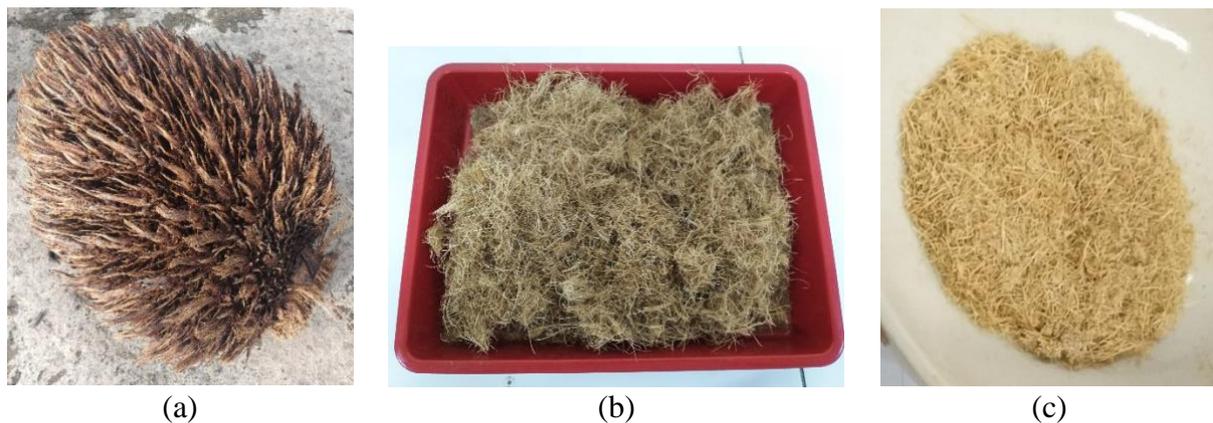


Figure 2. Freshly collected OPEFB (a), OPEFB after being cleaned and dried under the sun (b), OPEFB after being oven and ground into powder (c).

### 3.2. OPEFB Lignin Extraction and Oxalate Precipitation

The extraction of lignin from OPEFB powder is performed using the delignification method, aiming to separate lignin compounds from the lignocellulosic components of OPEFB powder. The OPEFB powder is delignified using a 4% oxalic acid dihydrate solution, and the process is carried out using an autoclave to accelerate the extraction process by increasing the reaction rate and lignin solubility in the acid solution. Oxalic acid serves as a good alternative organic acid catalyst to replace commonly used sulfuric acid for delignification ([Kalogiannis et al., 2018](#)). According to the report by [Bukhari et al. \(2021\)](#), oil palm trunk biomass containing lignocellulose was subjected to pretreatment using oxalic acid. The results showed that a 4% concentration of oxalic acid was more effective in breaking down the lignocellulosic structure compared to a 1% concentration. Additionally, the use of low-concentration oxalic acid required a longer delignification time. The autoclave conditions are set at a pressure of 1 atm and a temperature of 121°C for 90 minutes, as these conditions are optimal for extracting OPEFB in the oxalic acid dihydrate solution ([Suryadi et al., 2020](#)).

Lignin compounds contain hydroxymethyl groups, which form diketone and phenol products when oxidized with oxalic acid dihydrate. This reaction occurs through a neutral redox

process (without requiring changes in oxidation-reduction states) similar to the lignin breakdown process involving sodium formate-formic acid carried out by Stahl (Lindsay *et al.*, 2019). Based on the extraction process of 3 grams of OPEFB powder soaked in 18 mL of a 4% oxalic acid dihydrate solution, a liquid extract of  $8.17 \pm 0.47$  mL was obtained. Therefore, the obtained extract yield is 45.39%.

However, the extract still contains oxalate, necessitating a precipitation reaction to separate the oxalate into its salt form by reacting it with  $\text{Ca}(\text{OH})_2$ . The final products of the reaction are oxalate-free extract and calcium oxalate ( $\text{CaC}_2\text{O}_4$ ) salt. The two extracts have no significant visual difference, as both appear yellowish-brown and clear (see Figure 3). The precipitated  $\text{CaC}_2\text{O}_4$  salt appears as a fine white powder weighing  $399.5 \pm 3.2$  mg (see Figure 4).



Figure 3. OPEFB extract which still contains oxalate (a), oxalate-free OPEFB extract (b).



Figure 4.  $\text{CaC}_2\text{O}_4$  salt residue

Based on stoichiometric calculations, the weight of the  $\text{CaC}_2\text{O}_4$  salt precipitate should be 406.4 mg. Therefore, the difference of 6.9 mg between the experimental and stoichiometric calculations is not significantly different. Several factors can contribute to the variation between experimental and theoretical results, such as the sensitivity of the measurement instrument, the presence of materials that pass through the filter membrane during filtration, or incomplete

precipitation reactions due to temperature or reaction time variations. Hence, minimizing these factors and optimizing the experimental methods and conditions is important to achieve more consistent and accurate results.

### 3.3. Lignin Content Analysis of OPEFB Extract with UV-Vis Spectrophotometry

The OPEFB extract was determined using UV-Vis spectrophotometry to measure the lignin content in the samples. The result was 10,63 mg/g or 1.06%, as shown in Table 3. This method offers speed, accuracy, and ease of use advantages. Sodium lignosulfonate was used as the reference/standard for lignin. Sodium lignosulfonate is employed as an alternative for lignin calibration due to its relatively similar chemical structure and absorption characteristics to natural lignin. Based on determining the maximum wavelength, a value of  $\lambda_{\max}$  at 280 nm was obtained (see Figure 5a). This result is consistent with other studies reporting that the wavelength of lignin ranges from 225 to 287 nm (Lee *et al.*, 2013). According to the report by Lu *et al.* (2021), the highest absorbance of lignin compounds occurs between the wavelength range of 276 – 283 nm. Other research also supports the finding that the average maximum absorbance for lignin samples with higher purity is around the wavelength of 280 nm (Ruwoldt *et al.*, 2022).

Table 3. Results of analysis of lignin content of EFB extract at  $\lambda$  280 nm

Sample	Absorbance	Concentration (ppm)	Lignin content (mg/g)	Lignin content (%)
OPEFB Extract	0.844	1771.67	10.63	1.06

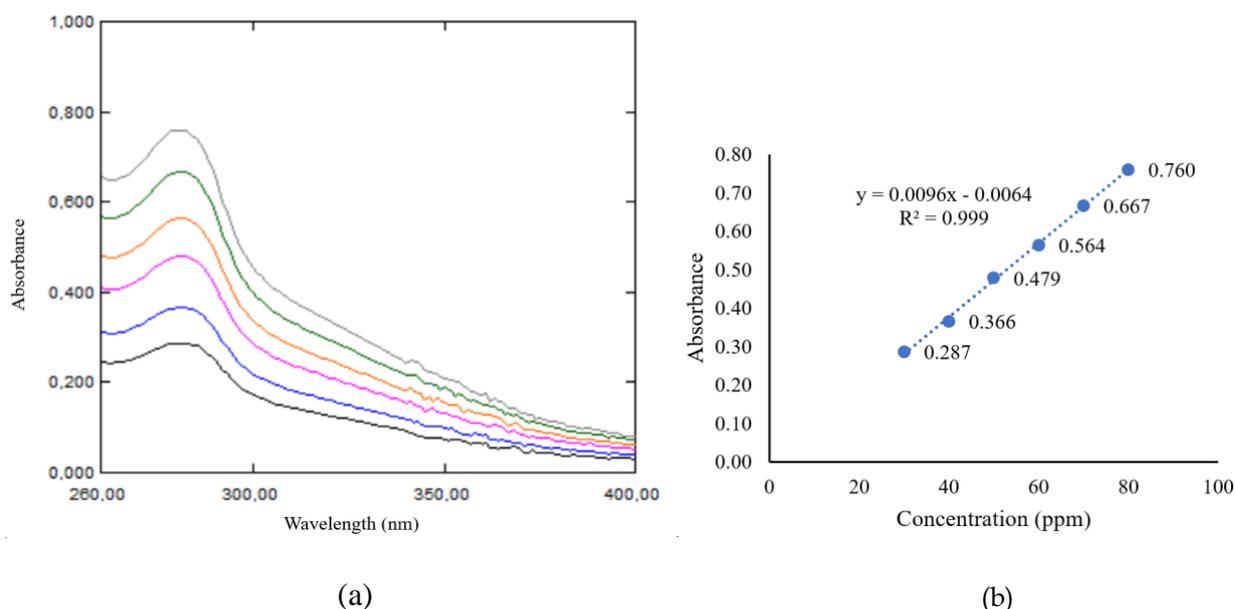


Figure 5. Lignin calibration spectrum by sodium lignosulfonate (a), lignin calibration curve based on the results of calibration spectrum readings (b)

Based on the calibration curve (see Figure 5b), it shows a linear relationship between lignin concentration and absorbance, resulting in linear regression (1).

$$y = 0,0096x + 0,0064 \quad (1)$$

Meanwhile, the coefficient of determination ( $R^2$ ) is 0.999, which indicates that the generated regression equation has a high level of accuracy and can be effectively used to measure lignin concentration accurately. The straight line on the graph demonstrates a high level of linearity and the accuracy of the UV-Vis spectrophotometric technique in measuring lignin concentration. The generated linear regression equation can calculate the sample's lignin content with a high accuracy level. The lignin determination spectrum is shown in Figure 6.

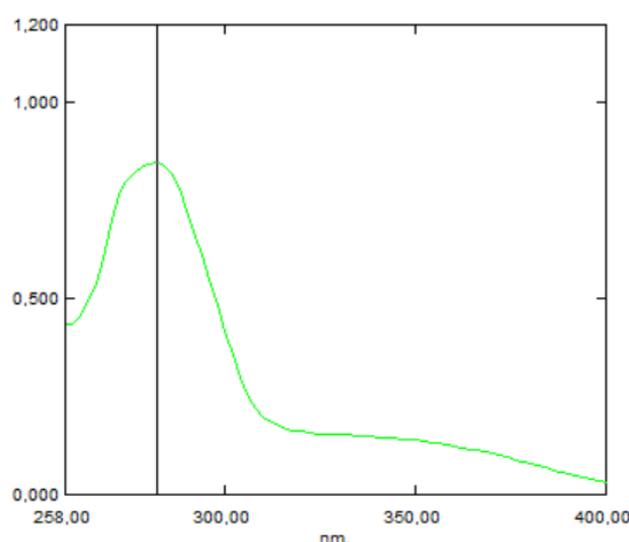


Figure 6. Spectrum analysis of lignin content of OPEFB extract

Table 4. The results of the antibacterial synergy test were based on the diameter of the clear zone of OPEFB lignin extract against *Staphylococcus aureus*

Disc	Information	Diameter of clear zone of lignin extract (mm)	
		With Oxalate	Free Oxalate
1	Negative control	8.13 ± 0.065*	N/A**
2	Single disc lignin extract	11.53 ± 0.735	N/A
3	Double disc lignin extract	16.65 ± 0.490	9.98 ± 0.408
4	Combination of lignin extract-amoxicillin	20.23 ± 0.490	15.83 ± 0.719
5	Double disc amoxicillin	21.13 ± 0.302	21.35 ± 0.572
6	Single disc amoxicillin	14.03 ± 0.245	14.23 ± 0.408

\* The negative control involved the use of a 4% oxalic acid solution (the solvent used in the delignification process)

\*\* The negative control involved the use of a 4% oxalic acid solution that had undergone precipitation of oxalate using  $Ca(OH)_2$ ; N/A: Not Applicable; All values shown in the table were replicated three times.

### 3.4. Antibacterial Synergy Test by The AZDAST Method

The antibacterial synergy test using the AZDAST method was conducted on the OPEFB lignin extract, wherein the extract was combined with the antibiotic amoxicillin to inhibit the growth of *Staphylococcus aureus* bacteria. Each disc was injected with 20  $\mu$ L of the test extract,

antibiotic, or negative control. Clear zones beyond the disc indicated the tested extract's or amoxicillin's activity in inhibiting bacterial growth. The size of the clear zones produced was measured using a millimetres calliper (mm). The results of this method demonstrated that the OPEFB lignin extract exhibited antibacterial activity against *S. aureus*.

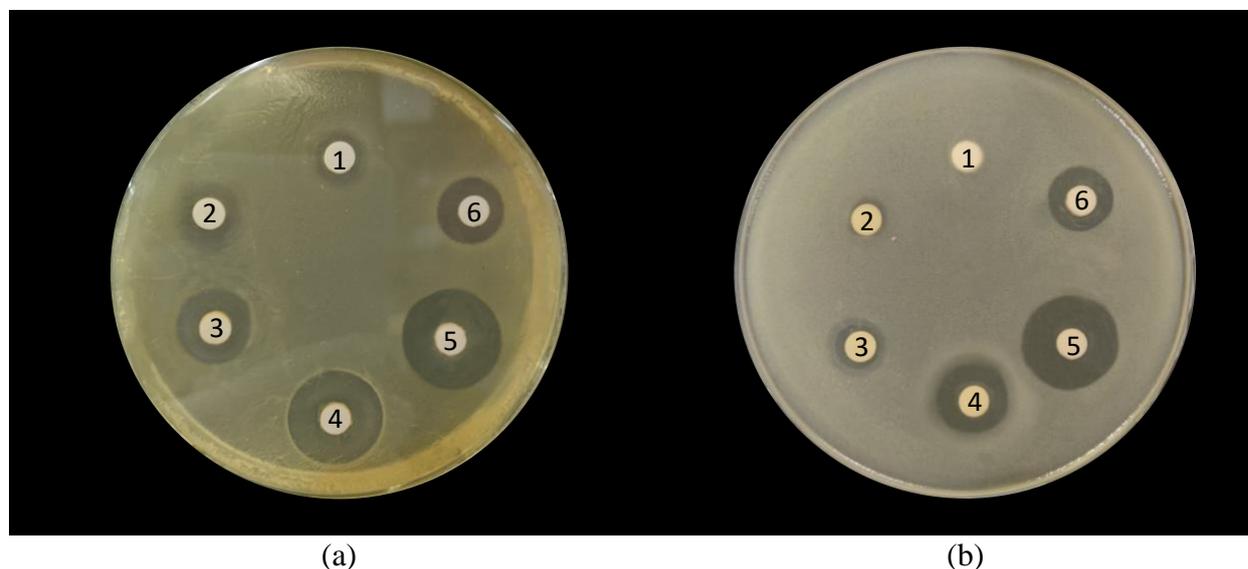


Figure 7. The clear zone in the antibacterial synergy test using the AZDAST method on *S. aureus* bacteria from OPEFB lignin extract which still contains oxalate (a), the clear zone from OPEFB lignin extract which is free of oxalate (b).

\*Remarks: 1. Negative control; 2. Lignin extract (single disc); 3. Lignin extract (double disc); 4. Combination of lignin extract and amoxicillin; 5. Amoxicillin (double disc); 6. Amoxicillin (single disc).

The results of the antibacterial synergy test can be seen in Table 4 and Figure 7. On disc 4 (a combination of lignin extract and amoxicillin), a clear zone with a diameter of 20.23 mm was formed on the lignin extract that still contained oxalate. In comparison, the oxalate-free lignin extract formed a clear zone with a diameter of 15.83 mm. The aim of comparing the inhibition zone of extracts that still contain oxalate with extracts that are free of oxalate is to confirm the effect of oxalate in the extract on antibacterial activity. However, based on statistical test results, it was found that the inhibition zone value of lignin extract containing oxalate was not significantly different compared to lignin extract without oxalate ( $p$ -value > 0.05). The difference in the size of the clear zones between the two lignin extracts can be influenced by the presence of oxalate compounds in the OPEFB extract, where the clear zone diameter of the lignin extract that still contained oxalate was larger compared to the clear zone diameter of the oxalate-free lignin extract. This observation is further supported by other data, as seen on disc 2 (lignin extract with a single disc) and disc 3 (lignin extract with double discs) of each lignin extract, where the lignin extract that still contained oxalate exhibited greater activity compared to the oxalate-free lignin extract. Furthermore, disc 1 (negative control) using a solvent containing oxalate also formed a clear zone. In contrast, no clear zone was formed when compared to disc 1 using a solvent without oxalate.

Based on the results, the oxalate compounds may affect clear zone formation, indicating the antibacterial activity of OPEFB lignin extract. However, the OPEFB lignin extract, which was oxalate-free, still exhibited antibacterial activity. In [Figure 7b](#), it can be observed that Disc 2 and Disc 3 can still form clear zones, although the clear zone in Disc 2 is very thin and cannot be measured using a calliper. Increasing the concentration of the extract can enlarge the clear zones formed, as seen in Disc 3.

The diameter of the clear zones formed by amoxicillin (Disc 5 and Disc 6) does not show significant differences between [Figure 7a](#) and [Figure 7b](#). This test found that the combination of OPEFB lignin extract and amoxicillin (Disc 4) can form larger clear zones compared to amoxicillin alone (Disc 6). However, it still does not surpass the clear zones formed by double-disc amoxicillin (Disc 5); this indicates that combining OPEFB lignin extract and amoxicillin has stronger antibacterial activity than amoxicillin alone. The statistical test results also showed that the inhibition zone value of the combination of lignin extract and amoxicillin was not significantly different from single amoxicillin, both extracts containing oxalate and free of oxalate (p-value > 0.05).

### 3.5. Data Analysis

The results of this study demonstrated that the lignin extract from oil palm empty fruit bunches, extracted with oxalic acid dihydrate, has the potential as an antibacterial agent against *S. aureus*. Referring to the interpretation criteria in [Table 1](#), as shown in [Table 5](#), it can be observed that the combined test results using the AZDAST method for the combination of OPEFB lignin extract with amoxicillin exhibited synergistic properties. However, further research is still needed to optimize the effectiveness of OPEFB lignin extract, especially in combination with antibiotics, and to check it against other bacteria while evaluating its potential toxicity.

Table 5. Interpretation types of combination test results with the AZDAST method

Lignin Extract	Disc	Results (mm)	Comparing	Interpretation of AZDAST combination test results
With oxalate	Negative control	8.13	-	AB > A & AB > B, and AB > AA even if AB < BB, it can be interpreted that the combination test is <b>synergistic</b> .
	Single disc lignin extract [A]	11.53	AB > A	
	Double disc lignin extract [AA]	16.65	AB > AA	
	Combination of lignin extract-amoxicillin [AB]	20.23	-	
	Double disc amoxicillin [BB]	21.13	AB < BB	
	Single disc amoxicillin [B]	14.03	AB > B	
Without oxalate	Negative control	0	-	AB > A & AB > B, and AB > AA even if AB < BB, it can be interpreted that the combination test is <b>synergistic</b> .
	Single disc lignin extract [A]	0	AB > A	
	Double disc lignin extract [AA]	9.98	AB > AA	
	Combination of lignin extract-amoxicillin [AB]	15.83	-	
	Double disc amoxicillin [BB]	21.35	AB < BB	
	Single disc amoxicillin [B]	14.23	AB > B	

#### 4. Conclusions

Based on the discussed results, it can be concluded that the oil palm empty fruit bunches (OPEFB) extracted with a 4% oxalic acid dihydrate solution at a temperature of 121°C and a pressure of 1 atm for 90 minutes obtained the lignin content of 10.63 mg/g or equivalent to 1.06%. In the antibacterial activity test against *Staphylococcus aureus*, it was found that the combination of amoxicillin and lignin extract containing oxalate had a larger inhibitory zone diameter compared to the combination of amoxicillin and lignin extract which was free of oxalate, but was not significantly different. The combination of lignin extract and amoxicillin had a larger inhibitory zone diameter than amoxicillin alone, indicating a synergistic effect of the combination. Unfortunately, both of them were still not significantly different (p-value > 0.05).

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