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SCALE-UP OF PHENOLIC COMPOUNDS PRODUCTION FROM *Styrax paralleloneurum* LEAVES AND EXTRACT PURIFICATION USING AMBERLITE XAD-2

Peningkatan Skala Produksi Senyawa Fenolik Daun *Styrax paralelloneurum* dan Purifikasi Ekstrak Menggunakan Amberlite XAD-2

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ABSTRACT

The current study aimed to determine the total phenolic compound content, antioxidant activity, and antibacterial activity of fermented Styrax leaf extract in 2000 mL volume. Fermentation by *Aspergillus niger* was carried out for 64 hours. After extraction, purification was conducted using Amberlite XAD-2 resin in ion exchange chromatography. The total phenolic content of the unpurified extract was 92.75 mg GAE.g^{-1}, while the purified extract was 146.67 mg GAE.g^{-1}. Antioxidant activity expressed in IC_{50} values was 31.71 ppm for the unpurified extract and 14.11 ppm for the purified extract. The antibacterial activity tested on *Staphylococcus aureus* and *Pseudomonas aeruginosa* showed an inhibition zone diameter of 31.43 mm and 28.2 mm, respectively for the unpurified extract, while for the purified extract 33.96 mm and 35.5 mm, respectively. It can be concluded that the purification of fermented Styrax leaf extract caused a stronger antioxidant activity and a very potent antibacterial activity.

Keywords: *Amberlite XAD-2, Aspergillus niger, ion exchange chromatography, Styrax paralelloneurum, submerged fermentation*

ABSTRAK

Penelitian ini bertujuan untuk menentukan kandungan senyawa fenolik, aktivitas antioksidan, dan aktivitas antibakteri ekstrak hasil fermentasi daun Styrax dalam volume kerja 2000 mL. Fermentasi oleh *Aspergillus niger* dilaksanakan selama 64 jam. Setelah ekstraksi, dilakukan purifikasi menggunakan resin Amberlite XAD-2 pada kromatografi pertukaran ion. Kandungan fenolik total dari ekstrak sebelum dimurnikan adalah 92,75 mg GAE.g^{-1}, sedangkan pada ekstrak setelah pemurnian adalah 146,67 mg GAE.g^{-1}. Aktivitas antioksidan yang dinyatakan dalam IC_{50} adalah 31,71 ppm untuk ekstrak yang belum dimurnikan dan 14,11 ppm untuk ekstrak yang telah dimurnikan. Aktivitas antibakteri, yang diuji terhadap *Staphylococcus aureus* dan *Pseudomonas aeruginosa*, berturut-turut menghasilkan diameter zona hambat 31,43 mm dan 28,2 mm untuk ekstrak yang belum dimurnikan, sedangkan untuk ekstrak yang telah dimurnikan berturut-turut adalah 33,96 mm dan 35,5 mm. Dapat disimpulkan bahwa purifikasi ekstrak daun Styrax menggunakan resin Amberlite XAD-2 menghasilkan aktivitas antioksidan dan antibakteri yang lebih tinggi.

Kata kunci: *Amberlite XAD-2, Aspergillus niger, fermentasi terendam, kromatografi penukar ion, Styrax paralelloneurum.*

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INTRODUCTION

Styrax parallelloneurum is a plant that grows widely in North Sumatra. The part of the tree that has been extensively researched and known to be useful is the frankincense sap. Frankincense sap is reported as having numerous potential bioactivities including antioxidant, antibacterial, antifungal, and anticancer (Sharif et al., 2016; Hacini et al., 2018; Hidayat et al., 2018; Hidayat et al., 2019; Gayatri et al., 2019). Styrax leaves also have the potential to produce bioactive metabolites such as glycosides, anthraquinones, saponins, flavonoids, and tannins as active antioxidants, anti-diabetic, and anti-inflammatory compounds. Besides that, Styrax leaves also have terpenoids which are antibacterial, antimicrobial, and anticancer compounds (Arbi, 2010). In the study by Naiborhu et al. (2023), antioxidant and antibacterial tests of fermented Styrax leaves were carried out on a laboratory scale.

The production of bioactive compounds from Styrax leaves can be enhanced by fermentation. Fermentation tends to damage the structure at the corner of the leaf cell wall, releasing bound phenolic compounds and forming bioactive compounds that can increase antioxidant activity (Mahardani & Yuanita, 2021). Several research groups have used submerged fermentation to produce higher phenolic content and antioxidant activity (Rafiquzzaman et al., 2015; Leonard et al., 2021). The fermentation of Styrax leaves can be done by the submerged fermentation method, where the experimental results showed an increase in total phenolic content by 3.96 times in the stationary phase compared to unfermented Styrax leaves. The study also tested antioxidant and antibacterial activities. The antibacterial activity of the fermented extract was tested against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. In the test against *S. aureus*, the diameter of the inhibition zone was 29.17 ± 0.76 mm, which is a very strong inhibition. Similarly, the test results on *P. aeruginosa* showed an inhibition zone diameter of 25.40 ± 0.36 mm, which includes a very strong inhibition. The antioxidant activity test using the DPPH method gave an IC_{50} value of 50.22 ± 2.26 ppm for the fermented Styrax leaf extract. This value shows an increase in antioxidant activity about 8.5 times higher than the IC_{50} value of unfermented Styrax leaf extract (Naiborhu et al., 2023).

Based on Naiborhu et al. (2023), it appears that Styrax leaves fermented by the submerged fermentation method have the potential as a medicinal raw material and it would be better if the production could be increased. The development of phenolic compound products from the laboratory scale to the industrial scale can be done by gradually increasing the production scale. In this study, a laboratory-scale production scale-up was carried out from a working volume of 150 mL (Naiborhu et al., 2023) to a working volume of 2000 mL. Increasing the working volume to 2000 mL is still included in the laboratory scale because the fermentation conditions are still the same as at the working volume of 150 mL. The consideration for using a working volume of 2000 mL is to determine whether there are significant differences in the concentration of phenolic compounds obtained. In addition, the working volume of 2000 mL confirmed the fermentation condition and concentration of the phenolic compound obtained before entering the pilot scale in subsequent research. Apart from increasing the concentration of phenolic compounds, this research also carried out the extract purification process using ion exchange chromatography, which is expected to increase the purity of phenolic compounds and their antibacterial and antioxidant activity.

MATERIALS AND METHODS

The main stages carried out in this research were the rejuvenation of microorganisms, fermentation, sample extraction, and purification using the ion exchange chromatography method. Total phenolic concentration, antioxidant activity, and antibacterial activity were measured on unpurified and purified samples. Each measurement was conducted in triplicate. Data were analyzed statistically using ANOVA.

Place and time of the research. The research was conducted from January until April 2022 at the Faculty of Biotechnology laboratory, Institut Teknologi Del.

Preparation of Styrax leaves. The Styrax leaves were obtained from the forest in Pollung District, Humbang Hasundutan Regency, North Sumatera. The leaves used were the whole leaves, with a width of about 2.0-2.5 cm and a length of 7.0-7.5 cm, which were the 5th to 10th leaves from the end of the twig. The leaves were washed using running water to remove impurities, dried in an oven at 50°C for 24 hours, then mashed using a grinder with a rotor speed of 1500 rpm, and filtered using a 60-mesh sieve.

Microorganism preparation. *A. niger*, *S. aureus*, and *P. aeruginosa* used in this research were obtained from the Laboratory of Microbiology, SITH, ITB. A. niger was rejuvenated on potato dextrose agar (PDA). Three loops were inoculated into 200 mL of yeast peptone glycerol (YPG) medium and incubated at 37°C using a shaker incubator. *A. niger* used as inoculum in the fermentation medium was in the logarithmic phase and aged 32 hours, with an absorbance of 0.103 at a wavelength of 600 nm and cell concentration of 0.00192 g/mL (Naiborhu et al., 2023). *S. aureus* and *P. aeruginosa* were also grown on a PDA medium, propagated on nutrient broth (NB) media, and incubated at 37°C. *S. aureus* used in the antibacterial test was in the logarithmic phase with an incubation time of 6 hours, while *P. aeruginosa* was in the logarithmic phase with an incubation time of 12 hours.

Submerged fermentation (SmF). The SmF procedure was carried out based on Naiborhu et al. (2023), but in this scale-up procedure, the amount of leaf powder and media volume is increased. A total of 200 g of Styrax leaf powder was added to 1800 mL of YPG. This was then sterilized at 121°C for 15 minutes and allowed to cool at room temperature. Up to 10% (v/v) *A. niger* inoculum was added to the fermentation medium to give a final volume of 2000 mL. The fermentation medium was stirred with a magnetic stirrer and incubated at 37°C. The fermentation process was stopped when *A. niger* reached the stationary phase after 64 hours of incubation. The fermentation process was carried out in three repetitions. Extraction was carried out by the maceration method by adding 2600 mL of 70% ethanol to the fermented media and stirring for 24 hours at room temperature. The extract was filtered through gauze and concentrated at 40°C using a rotary evaporator.

Purification. The purification procedure carried out in this research was based on Trikas et al. (2017). Purification of Styrax leaf extract was carried out using the ion exchange chromatography method and Amberlite XAD-2 resin. The resin was soaked in methanol overnight and then placed in a plastic syringe column with a resin volume of approximately 0.75-0.85 cm^3. After filling the column with resin, water was poured into the column and the resin was equilibrated with 4x BV (bed volume) of a mixture of extraction solvents, namely 70% ethanol and water (1:1). The resin was pre-treated by running 2x BV of 4% v/v HCl through the column and then rinsing with 4x BV of deionized water. Deionized water is used because it contains no minerals or ions, so there is no binding between chloride ions and ions in the water. Therefore, rinsing the column with deionized water can remove any chloride ions still present on the surface of the resin. Up to 2x BV 4% w/v NaOH was then injected into the column and rinsed with 5x BV deionized water. After the pre-treatment step, 1.3x BV of Styrax leaf extract samples were injected into the column, then the column was rinsed with 1.3x BV of deionized water and then eluted with 1.3x BV of 3% v/v formic acid. Purification was carried out for each of the three sample extracts, and any effluent was collected and used for the next stage of the assay.

Determination of total phenolic content. Total phenolic content was determined according to Naiborhu et al. (2023). Gallic acid standard curves were constructed by measuring the absorbance of 8 concentrations of gallic acid standard solutions at concentrations of 0-100 ppm. Up to 0.01 g of gallic acid was placed in a 100 mL volumetric flask and 70% ethanol was added to the mark, and dilutions were made to obtain 8 standard solution concentrations. From each concentration of the gallic acid standard solution, 1 mL was taken and placed in a 10 mL volumetric flask, to which 1 mL of Folin-Ciocalteau reagent was added. When homogeneous, 4 mL of 1N Na_2CO_3 was added. The absorbance of the solution was measured at a wavelength of 725 nm. The absorbance value (Y-axis) was

plotted against the standard concentration of gallic acid (X-axis) to obtain a linear regression equation Y = 0.0232X + 0.5079 with a value of R^2 = 0.995. The measurement of the total phenolic content of Styrax leaf extract was performed by mixing 1 mL of Folin-Ciocalteau reagent with 1 mL of unpurified Styrax leaf extract or purified Styrax leaf extract. Once homogenized, 4 mL of 1N Na_2CO_3 was added and the absorbance was measured at a wavelength of 725 nm. The total phenolic content of the Styrax leaf extract was measured in triplicate. The absorbance value is entered in the linear regression equation to obtain the total phenolic extract concentration (mg GAE.g^{-1}).

Antioxidant activity assay. The antioxidant activity assay was performed according to the method of Naiborhu et al. (2023). The sample extract was diluted to obtain 5 concentrations namely 50, 100, 150, 200, and 250 ppm. Each concentration was made triplicate. 2 mL of sample solution was added to 2 mL of 50 ppm DPPH (2,2-diphenyl-1-picrylhydrazyl) solution and then incubated at 25°C for 30 minutes. The absorbance was measured at a wavelength of 517 nm. A blank solution consisting of 2 mL of 70% ethanol and 2 mL of 50 ppm DPPH was used for absorbance measurements. The absorbance data of each concentration is entered into equation (1) to obtain the % inhibition value, which represents the antioxidant activity.

$$\%inhibition = \frac{Absorbance_{blank} - Absorbance_{sample}}{Absorbance_{blank}} \; x \; 100\% \qquad (1)$$

The value of each % inhibition was plotted against the concentration value and the linear regression equation Y = aX + b was obtained. From the sample extract without purification, a regression equation Y = 0.8959X + 49.433 is obtained with a value of R^2 = 0.994, while from the sample extract resulting from purification, a regression equation Y = 4.3742X + 48.761 is obtained with a value of R^2 = 0.9875. Based on the linear regression equations that have been obtained, the 50% inhibition concentration (IC_{50}) value is calculated by substituting the 50% as the Y value and the X value obtained is the concentration of the extracted sample which has the activity of an antioxidant required to scavenge 50% of the initial DPPH radicals. The lower the IC_{50} value, the more potent is the sample at scavenging DPPH and it means a higher antioxidant activity.

Antibacterial activity test. The antibacterial activity test was performed according to the method of Naiborhu et al. (2023). A total of 14.0449 g of nutrient agar (NA) media was dissolved in 500 mL of distilled water and then sterilized at 121°C for 15 minutes. Sterile media was poured into 6 Petri dishes and allowed to cool at room temperature. Antibacterial activity was tested against *S. aureus* and *P. aeruginosa* in the logarithmic phase. A total of 0.1 mL of bacterial suspension was spread evenly over the surface of the solid NA medium using an L-rod. Each sterile paper disc was dipped in 5 µL of unpurified fermented Styrax leaf extract, the purified extract, the antibiotic nebacetin as a positive control, and 70% ethanol of sample solvent as a negative control, and then placed on the surface of the agar. The agar medium was incubated at 37°C for 24 hours, and then the diameter of the clear zone formed around the disc paper was measured using a caliper.

RESULTS

The total phenolic concentration was determined on the submerged fermented Styrax leaf extract purified by the ion exchange chromatography method and the unpurified extract. The results of the total phenol concentration measurement shown in Figure 1 showed a significant increase in the purified extract (146.84 mg GAE.g^{-1}) compared to the unpurified extract (92.75 mg GAE.g^{-1}), with an increase of 58.31%.

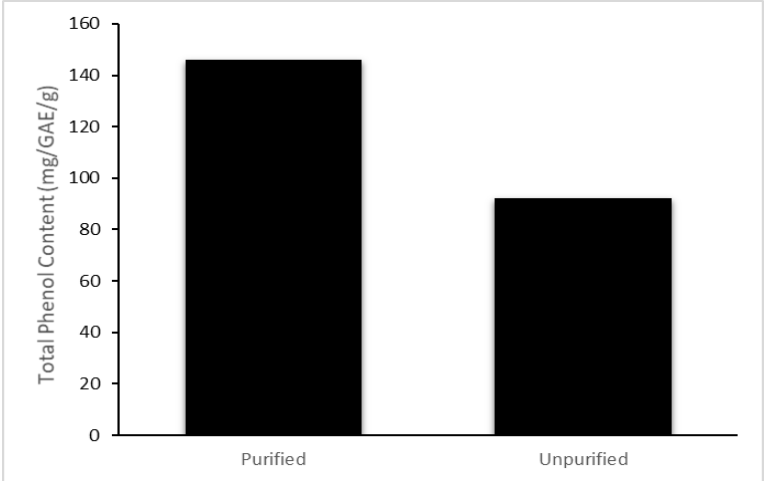

Figure 1. Total phenolic content of unpurified and purified extract of fermented frankincense leaves.

The results of the antioxidant activity test are expressed in IC_{50} values. The IC_{50} value is the concentration of the extracted sample which has the activity of an antioxidant required to scavenge 50% of the initial DPPH radicals. The IC_{50} value was obtained from the linear regression equation, which states the relationship between the concentration of the sample extract and the percentage of inhibition of radicals (Sadeli, 2016). The test results showed that the IC_{50} value of the purified extract sample was significantly lower (14.1174 ppm) than the IC_{50} value of the unpurified extract sample (31.7159 ppm), where the lower the IC_{50} value, the stronger the antioxidant activity. The results of the IC_{50} values obtained are shown in Figure 2. From the IC_{50} values in each extracted sample, it can be categorized as having strong antioxidant properties based on categories stated in Phongpaichit et al. (2007).

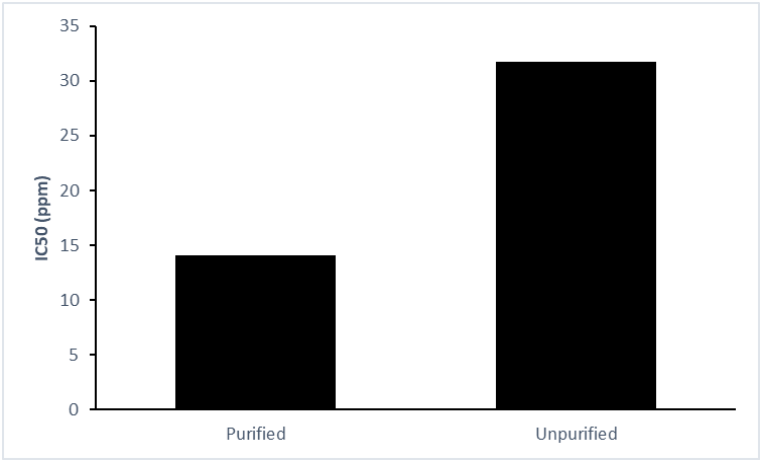

Figure 2. Antioxidant activity (IC_{50}) of purified and unpurified extract of fermented frankincense leaves.

Figure 3A showed the average diameter of the inhibition zone of unpurified Styrax leaf extract, purified extract, positive control Nebacetin, and negative control 70% ethanol for *S. aureus*, which were 31.43 mm, 33.96 mm, 38.56 mm, and 0.0 mm, respectively. Figure 3B shows the inhibition zone diameters of unpurified Styrax leaf extract, purified extract, positive control Nebacetin, and negative control 70% ethanol for *P. aeruginosa*, namely 28.20 mm, 31.43 mm, 24.23 mm, and 0 mm. The results of the antibacterial test on *S. aureus* and *P. aeruginosa* showed that unpurified and purified Styrax leaf extract had very strong inhibitory effects according to the categories stated in Surjowardojo et al. (2015).

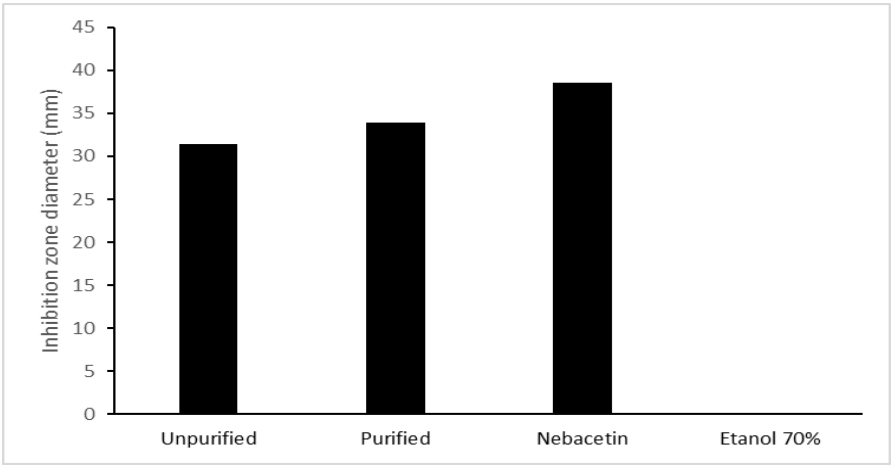

Figure 3A. Antibacterial activity of unpurified and purified extract of fermented frankincense leaves to *Staphylococcus aureus*.

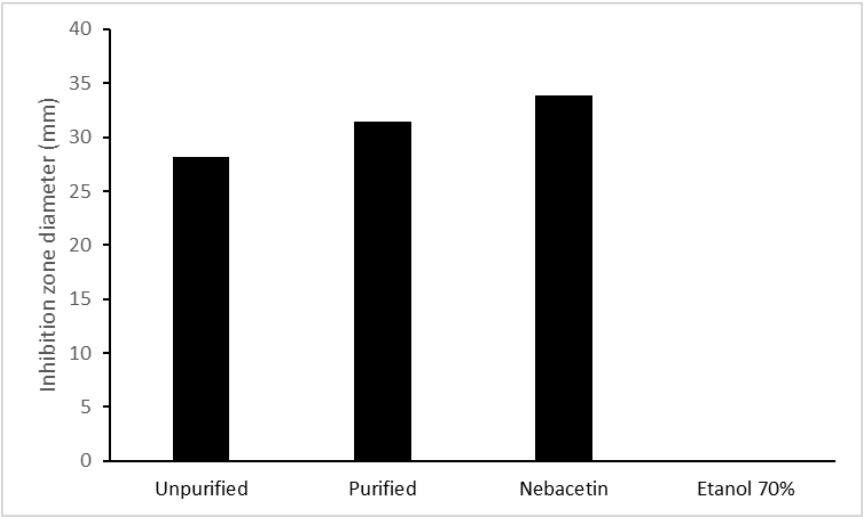

Figure 3B. Antibacterial activity of unpurified and purified extract of fermented frankincense leaves to *Pseudomonas aeruginosa*.

DISCUSSION

Total phenolic content. Figure 1 showed a significant increase in the total phenol concentration in the purified extract sample compared to the total phenol concentration in the unpurified extract sample. The increase in the total phenol concentration in Styrax leaves was caused by several factors, including the metabolic processes of the microorganisms during fermentation, the solvents used in the maceration stage, and the purification process. The first cause, which is the fermentation process, is a method that can be used to increase the bioactive components present in plants. According to Zhao et al. (2021), fermentation is useful for inducing degradation in plant cell walls, which results in the formation of bioactive components. In the fermentation process, the role of microorganisms is very important in increasing the formation of phenolic compounds, especially in submerged fermentation, which involves microbial growth using media with high humidity. Therefore, this fermentation is suitable for the use of microbes such as fungi (Rousta et al., 2021). A. niger used in this fermentation, can produce or enhance bioactive compounds because it can produce enzymes such as glycosides and hydrolases, enzymes that can degrade polysaccharides and destroy plant cell walls (Blumer et al., 2023). This enzyme can catalyze the

hydrolysis of β-glucoside bonds from phenolic compounds and increase the concentration of free polyphenolic compounds present in plants. Styrax leaves contain phenolic compounds, and the total phenolic concentration can increase due to the increase in phenolic group compounds during the fermentation process (Ismail et al., 2019).

The production of various types of enzymes by A. niger during the fermentation process can damage plant cell walls and cause the release of bioactive compounds, including phenolic compounds present in plants, into the fermentation medium. Research conducted by Naiborhu et al. (2023) showed that submerged fermentation can increase phenolic content because the submerged fermentation method is very effective in increasing secondary metabolites. By increasing the working volume to 2000 mL in this study, the total phenol concentration was 92.75 mg GAE.g^{-1} compared to 40.83 mg GAE.g^{-1} in a previous study by Naiborhu et al. (2023) using a working volume of 150 mL. Research conducted by Adeyi et al. (2022) also showed that increasing culture volume will affect the increased yield of *Carica papaya* extract. This indicates that an increase in production scale leads to an increase in the total yield of bioactive compounds. The increase in yield of secondary metabolites during the increasing scale of plant tissue culture work is due to changes in the culture condition or shifts in growth (Filova, 2014).

The second cause is the ethanol solvent used in the maceration stage of fermented Styrax leaf extract. Ethanol solvent is very effective in the extraction of Styrax leaves, resulting in high levels of total phenol. This is consistent with the research of Jimenez-Moreno et al. (2019) and Lohvina et al. (2022), that ethanol solvent has a high influence on the extraction yields of phenolic compounds. Ethanol as a solvent will extract phenolic compounds in plants because polar compounds will dissolve compounds that are also polar.

The third cause is the method of purification. In Figure 1, it can be seen that there was an increase of 58.31% in the total phenolic concentration in the extract samples that underwent a purification process compared to the extract samples that were not purified. In this study, the purification of phenolic compounds was carried out using Amberlite XAD-2 resin, which is a polystyrene copolymer resin that has hydrophobic cross-links and belongs to a macroporous resin that has a high adsorption capacity. Macroporous resin is derived from a non-polar styrene-divinyl-benzene (SDVB) structure. The chemical structure of the resin greatly influences the adsorption capacity of phenolic compounds. The pore surface area factor can affect the adsorption capacity (Park & Lee, 2021; Kodjapashis et al., 2023), where a larger pore surface area provides a better opportunity to adsorb phenolic compounds. Another factor that contributes to the adsorption capacity is the pore structure of the resin, as solutes must migrate through the pores to the absorbent surface. Therefore, the pore size of the resin must be large enough to accommodate phenolic compounds, but small enough to prevent other larger phenolic compounds from entering. The pore size of Amberlite XAD-2 resin for adsorption and desorption of phenolic compounds is 90Å. Amberlite XAD-2 resin is characterized by its unique macro reticular porosity, broad pore size distribution, and large surface area, as well as a chemically homogeneous non-ionic structure that distinguishes it from most other adsorbents. As a result, these resins are widely used to adsorb dissolved organic compounds from water and organic solvent streams, typically in cyclic column operations (Avino et al., 2011).

Antioxidant activity. Increasing the concentration and activity of phenolic compounds, which are antioxidants, can be achieved through a fermentation process (Sulasiyah et al., 2018; Nurmiati & Wijayanti, 2018). Enzymes produced in the fermentation process, such as glucosidase, tannase, or xylase, play a role in the degradation of the plant cell wall matrix, thereby facilitating the extraction of phenolic compounds (Hur et al., 2014). One of the enzymes from the fungus *Aspergillus niger*, β-glucosidase, can catalyze the hydrolysis of glycosidic linkages in alkyl and aryl β-D-glucosides. These enzymes help break the bonds between the sugars and release the glycosides, thereby releasing free phenolic or phenolic aglycone

groups (Zhang et al., 2021; Muradova et al., 2023). According to Tosun et al. (2009), phenolic compounds can scavenge free radicals due to their hydroxyl groups. The antioxidant activity of phenolic compounds depends on the number and relative orientation of the hydroxyl groups to functional groups such as CO_2H, CH_2CO_2H, or $(CH)_2CO_2CH$, which withdraw electrons, so that the higher the total phenol content, the higher the antioxidant activity of an extract. Figure 2 shows that the fermentation and purification processes using the ion exchange chromatography method can increase the antioxidant activity by 55.49%.

Antibacterial activity. Figures 3A and 3B showed that there was an increase in antibacterial activity in purified samples of Styrax leaf extract, as indicated by the larger average diameter of the inhibition zone, compared to unpurified samples of Styrax leaf extract, against *S. aureus* and *P. aeruginosa*. From the research conducted on *S. aureus* using samples of unpurified Styrax leaf extract, the average diameter of the inhibition zone was 31.43 mm, while for *P. aeruginosa* it was 28.2 mm. Samples of purified Styrax leaf extract produced an average inhibition zone diameter of 33.96 mm for *S. aureus*, while for *P. aeruginosa* it was 31.43 mm. The diameter of the inhibition zone obtained for *S. aureus* and *P. aeruginosa* was included in the very strong category because the average diameter of the inhibition zone was greater than 21 mm (Surjowardojo et al., 2015). The increase in the diameter of the inhibition zone could be due to the increase in the number of phenolic compounds in the fermented Styrax leaves to the stationary phase. According to Dey et al. (2014), this is because the fermentation process converts the phenolic compounds into a form that is more soluble in ethanol.

According to Siahaan et al. (2015), the diameter of the inhibition zone resulting from the extraction process using ethanol as a solvent is related to the components that can be extracted. Polar ethanol can attract other polar compounds such as phenols, saponins, alkaloids, and terpenoids. These compounds are phenolic compounds that have antioxidant and antibacterial activity. Therefore, ethanol was used as a solvent in this study to dissolve phenolic compounds, especially phenolic compounds that had been converted to a more soluble form by the fermentation process. Phenolic compounds in plants have broad-spectrum antibacterial activity against Gram-positive and Gram-negative bacteria. Phenol derivatives interact with bacterial cells through absorption processes involving hydrogen bonds (Tursiman et al., 2012) and interfere with the work in the cytoplasmic membrane, including interfering with active transport and proton strength. Phenol causes hyperacidification, disrupting transmembrane permeability and ion channel proteins, as well as signaling pathways between cells (Mantzourani et al., 2019). Based on the test results, the antibacterial activity showed a consistent relationship with the total phenolic content of the Styrax leaf extract, where the higher the total phenolic content of the Styrax leaf extract, the higher the inhibitory ability of the extract against *S. aureus* and *P. aeruginosa*.

The antibacterial activity tests performed show that phenolic compounds can inhibit the growth of *S. aureus* bacteria by producing a larger diameter of inhibition zone compared to *P. aeruginosa* in purified and unpurified samples of Styrax leaf extract. This is because *S. aureus* is a Gram-positive bacterium that has a peptidoglycan that reaches a thickness of 80 nm, but does not have an outer membrane. The number of peptidoglycan layers in Gram-positive bacteria is 40 layers, which is 90% of all cell wall components (Madigan et al., 2012). Gram-positive bacteria have a sensitivity level that tends to be higher than Gram-negative bacteria because their cell wall structure is simpler, so antibacterial compounds enter the cells more easily (Hamidah et al., 2019). The mechanism of antibacterial action of phenolic compounds on the cell walls of Gram-positive bacteria *S. aureus* is by preventing the incorporation of N-acetylmuramic acid bonds into the mucopeptide structure, which would create rigidity in the cell wall so that the formation of the bacterial cell wall is disrupted and does not form completely. As a result, the bacteria lose their rigid cell wall, leaving the cell membrane vulnerable to damage and leakage. According to Madigan et al. (2012), the mechanism of action of antibacterial compounds against *S.*

aureus is to inhibit peptidoglycan biosynthesis in the transpeptidase reaction, which ultimately causes cell lysis.

The antibacterial activity test on *P. aeruginosa* resulted in a smaller diameter of the inhibition zone compared to the diameter of the inhibition zone on *S. aureus*. This is because *P. aeruginosa* is a Gram-negative bacterium with a non-simple membrane. The relatively complex cell wall of Gram-negative bacteria plays an important role as a molecular barrier that can prevent the loss of intracellular proteins and reduce access to enzymes and other hydrolytic agents, especially hydrophobic antibiotics (Pinto et al., 2017). The outer membrane of *P. aeruginosa* can inhibit the penetration of the antibiotic and the efficient removal of the antibiotic molecule via an efflux pump before it acts on its target. The cell membrane of *P. aeruginosa* has open channels, which are very rare compared to other Gram-negative bacteria, and the presence of an efflux pump can combat the influx of antibacterial agents that have managed to enter through the cell membrane (Alhazmi, 2018). The permeability of the outer cell membrane of Gram-negative bacteria is also influenced by porins. Porins regulate the movement of molecules into the periplasmic space for transport across the cytoplasmic membrane. Porins can cause passive diffusion of low molecular weight hydrophilic components but are impermeable to large molecules (Samanta et al., 2018). The number of small porins will affect the penetration of antibacterials, especially those that are relatively large in the content of most botanical active compounds.

The antibacterial activity test used the antibiotic Nebacetin as a positive control. Nebacetin is classified as an antibacterial compound containing neomycin and bacitracin. Neomycin, an aminoglycoside, is more capable of inhibiting the growth of Gram-negative bacteria, but can also inhibit Gram-positive bacteria by irreversibly binding to the 30S ribosomal subunit, thereby inhibiting bacterial protein synthesis (Brewer, 1977). Bacitracin is a polypeptide produced by a strain of *Bacillus licheniformis* that can inhibit Gram-positive bacteria by inhibiting the dephosphorylation of C55-isoprenyl pyrophosphate, which is important for the regeneration of lipid carriers required for the synthesis of cyclic peptidoglycan. Therefore, bacitracin can disrupt the membrane structure either by removing lipids or by direct penetration. Nebacetin effectively eliminates *S. aureus*, *S. epidermis*, *P. aeruginosa*, and *Escherichia coli* bacteria *in vitro* (Bisdas et al., 2012).

CONCLUSION

The scale-up volume of submerged fermentation of *Styrax paralelloneurum* leaves by *Aspergillus niger* and purifications of its extracts using Amberlite XAD-2 resin, significantly increased total phenolic content, causing a stronger antioxidant activity, as well as causing a very potent antibacterial activity to *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The prospects of the purified extract are vast enough for further research and to be implemented as a raw material for medicine.

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