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Research article

Quantification of antioxidant and antimicrobial properties of oil palm empty fruit bunch lignin based on organosolv extraction

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Abstract

Importance of the work: Lignin from an oil palm empty fruit bunch (OPEFB) sample was extracted using an organosolv technique to characterize the antioxidant and antimicrobial properties.

Objectives: To optimize the OPEFB extraction conditions for lignin using different mixtures of organic acid solution and extraction times.

Materials & Methods: OPEFB samples were pretreated using dilute sulfuric acid, followed by organosolv extraction. The most effective extraction conditions were identified for producing lignin. This lignin was characterized based on Fourier-transform infrared (FTIR) spectroscopy and various properties, including molecular weight, particle size, total phenolic content (TPC) and antioxidant and antimicrobial activities.

Results: The study involved pretreating OPEFB with 8% (volume per volume) sulfuric acid, then extracting with different mixtures of organic acid containing formic acid (FA), acetic acid (AA), and water (H_2O) and using different extraction durations. The optimal extraction conditions were achieved by combining FA/AA/H_2O in the ratio 30:60:10% (volume per volume per volume) and maintaining it for 60 min. The mean (± SD) effectiveness of lignin extraction was 44.1 ± 0.06%. The purity of the extracted lignin was 92.2 ± 0.21%, comprising 91.0 ± 0.22% acid-insoluble lignin and 1.16 ± 0.02% acid-soluble lignin. The produced lignin had a small molecular size and most functional groups were phenol hydroxyl groups, which affected the antioxidant and antimicrobial activities.

Main finding: The organosolv technique showed potential for lignin extraction from OPEFB. This method not only uses greener chemicals compared to conventional approaches but also identified antioxidant and antimicrobial activities as a way of adding value to agricultural waste materials.

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Introduction

Oil palm is a cash crop that grows well in tropical countries near the equator, with extensive plantings in Southeast Asia, Malaysia, Indonesia and Thailand (Noah, 2022). About 2 million t of oil palm empty fruit bunches (OPEFBs) are generated as waste annually in Thailand (Noah, 2022). Most oil palms are used as raw material for producing cooking oil, animal feed and biodiesel. However, a major portion of the OPEFB produced remains as industrial waste, with most being burned to generate fuel for factories (Khomlaem et al., 2023). This is considered a low-value implementation because OPEFB is a lignocellulosic biomass, with the main components being cellulose, hemicellulose and lignin in various amounts, depending on the season, farmland and climatic conditions (Medina et al., 2015). Cellulose, hemicellulose and lignin have the potential to be developed as value-added materials (Madurwar et al., 2013).

OPEFB, as a form of lignocellulosic biomass, is comprised of 24–65% cellulose, 21–34% hemicellulose and 14–31% lignin (Chang, 2014). Enzymatic hydrolysis breaks the cellulose and hemicellulose into glucose and xylose, which can be further converted into value-added products (Zulkiple et al., 2016). However, the resistance of OPEFB to decomposition makes it challenging to obtain carbohydrate polymers. In general, several factors restrict the ability of lignocellulosic biomass to be digested by enzymes, such as biomass particle size, surface area, acetyl groups bound to hemicellulose, degree of polymerization, cellulose crystallinity and the presence of lignin. (Kumar and Wyman, 2009). Therefore, pretreatment is necessary to access the sugar polymers and open the lignocellulose structure of the OPEFB for further valorization.

Lignocellulosic biomass can be pretreated mechanically, chemically, biologically or through a combination of these methods (Kumar and Sharma, 2017). Applying acids or bases for chemical pretreatment is an effective pretreatment to increase the digestibility of lignocellulosic biomass (Chen et al., 2017). For example, in acidic pretreatment, the lignocellulosic structure is primarily deconstructed by dissolving the hemicellulose, leaving lignin and cellulose in the remaining solid (Li et al., 2012). On the other hand, the lignin linkages can be easily broken by alkaline pretreatment, which also improves the solubilization of the polymer (Kim et al., 2016). Although lignin can be successfully removed using this technique, recovering the lignin dissolved in the liquid is challenging. Consequently, recovering lignin in solid form from lignocellulose by using acid as a pretreatment approach is effective before lignin extraction.

Lignin is one of the most abundant renewable resources of lignocellulosic biomass (Ayyachamy et al., 2013). Lignin is an aromatic biopolymer with indefinite crystal shapes and is responsible for the structural strength between fibers Lee et al. (2014). Lignin molecules are large and complex, consisting of three monomers containing coniferyl alcohol, sinapyl alcohol and p-coumaryl alcohol that are collectively known as monorinol and are joined by various linkages such as β–O–4, α–O–4, β–5 and β–β (Ayyachamy et al., 2013; Akbari et al., 2020). The main functional groups of lignin are hydroxyl, carbonyl and carboxyl groups that provide various properties, such as solubility in water (El Mansouri and Salvadó, 2006).

The extraction of lignin can be performed using various techniques. The general method is alkaline extraction, which uses sodium hydroxide or potassium hydroxide as solvents (Kim et al., 2016; Thanapimmetha et al., 2023). Alternatively, lignin extraction can use the organosolv technique that provides high-purity lignin, with a low ash content that is utilizes easy-to-reuse solvents, including ethanol, acetone, formic acid and acetic acid (Lora, 2008; Alwadani, 2017). In addition, it is possible to enhance high-purity lignin if the raw materials are prepared adequately before extraction.

The current study investigated an organosolv technique of extracting lignin from OPEFBs after pretreating the OPEFBs with dilute sulfuric acid. The extraction process was influenced by two key factors: the composition of the solvent mixture, comprising a mixture of formic acid (FA), acetic acid (AA) and water (H_2O) and the duration of the extraction. The lignin extraction aimed to evaluate the lignin purity through the combined assessment of acid-insoluble lignin (AIL) and acid-soluble lignin (ASL). This lignin purity and efficiency evaluation was essential in determining the optimal extraction conditions. Following this, extensive characterization of the obtained lignin was carried out using Fourier-transform infrared (FTIR) spectroscopy, with evaluation undertaken of its molecular weight, particle size, total phenolic content (TPC) and antioxidant and antimicrobial properties.

Materials and Methods

Chemicals and materials

All chemicals used in this study were analytical grade of sulfuric acid, FA, AA, acetone, sodium carbonate, dimethyl sulfoxide (DMSO), Folin-Ciocalteu reagent, gallic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH).

Suksomboon Vegetable Oil Co., Ltd., Nong Yai, Chonburi, Thailand, provided the OPEFBs used as the substrate. The OPEFB was cleaned and sun-dried for 2 wk to a constant weight and was then stored in dry plastic containers. Next the OPEFB was cut, ground and sieved through a 40–60 mesh. The chemical composition of the untreated OPEFB was analyzed according to TAPPI standards (Sluiter et al., 2012). The overall exaction process of the produced lignin from OPEFB is shown in Fig. 1.

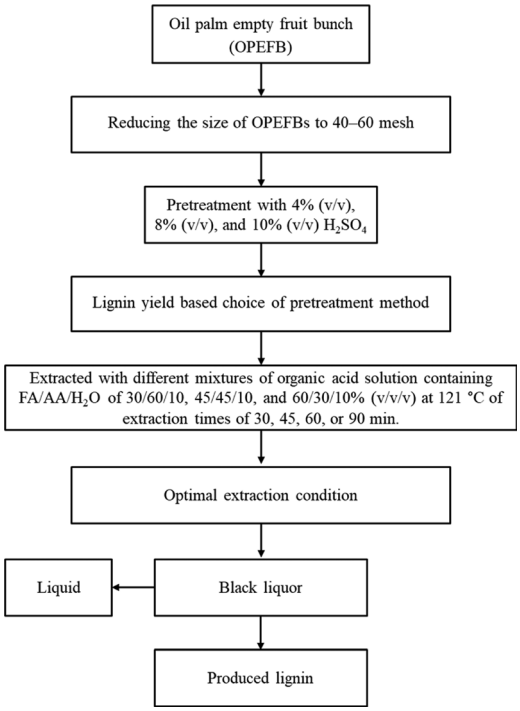

Fig. 1 Schematic representation of lignin production from oil palm empty fruit bunches (OPEFBs) using organosolv technique

Pretreatment of oil palm empty fruit bunch

The OPEFB sample was treated with dilute acid in various concentrations of 4% (volume per volume; v/v), 8% (v/v) or 10% (v/v) sulfuric acid (H_2SO_4) solution. A 10% (w/v) solid (dry OPEFB)-to-liquid (each dilute acid) ratio was prepared and autoclaved at 121°C for 60 min at 103.4 kPa, according to Thanapimmetha et al. (2019). The solid residue was recovered and neutralized by washing with deionized (DI) water. Then, it was dried at 105°C for 2 hr, after which its chemical components were analyzed according to the TAPPI standard (Sluiter et al., 2012).

Lignin extraction using organosolv technique

Lignin extraction using the organosolv technique involved organic solvents (formic and acetic acids). The reactions were conducted in an autoclave at a constant temperature of 121°C, with varying extraction durations of 30, 45, 60 or 90 min. The experiments encompassed three distinct mixtures of organic acid solutions, each consisting of formic acid, acetic acid and water in different proportions (30/60/10% volume per volume (v/v/v), 45/45/10% (v/v/v), and 60/30/10% (v/v/v)). The pH of the resulting black liquors from each extraction was neutralized using DI water and subsequently centrifuged at 6,500 rpm for 15 min. The produced lignin was dried at 105°C for 2 hr until a constant weight was achieved. Subsequently, the produced lignin pellets were subjected to analysis to determine the efficiency of lignin extraction based on dry biomass, as defined in Equation 1:

$$\text{Efficiency of lignin extraction} = \frac{\text{Weight of produced lignin}}{\text{Weight of lignin on treated OPEFB}} \times 100\% \quad (1)$$

Lignin purity, calculated from the summation of acid-insoluble lignin (AIL) and acid-soluble lignin (ASL), was assessed to identify the optimal extraction conditions.

Characterization of the produced lignin

Characterization of the produced lignin was carried out using FTIR spectroscopy, gel permeation chromatography (GPC) and dynamic light scattering (DLS).

Fourier-transform infrared spectroscopy

The chemical structure and functional groups of the produced lignin were analyzed using an FTIR spectrometer (Bruker; Billerica, MA, USA). The FTIR spectra were recorded in the 400–4000 cm^{-1} wave number range, as described by Chin et al. (2021).

Gel permeation chromatography analysis

The molecular weight of the produced lignin was analyzed using GPC (Waters e2695 separations module; Waters Corporation; Milford, CT, USA) using solvent tetrahydrofuran as the injection eluent at 35°C, with a flow rate of 1 mL/min, volume injected of 20 µL and with a refractive index detector. Calibration curves were constructed with polystyrene standards, according to Lange et al. (2016).

Dynamic light scattering measurement

A Zetasizer instrument (Nanoseries model S4700; Malvern Instruments; Worcester, UK) was utilized for particle size analysis (Lim et al., 2016). Approximately 2 mL of the water-based colloidal lignin suspension, characterized by a viscosity of 0.8872 *Cp*, was introduced into a cuvette containing lignin concentrations of 0.01 wt% for the sample. Particle size measurements were conducted at 25°C and the data were analyzed using a refractive index of the sample of 1.33. The sizes of the particles were determined using an intensity-weighted average (Z-average) of scattered light and presented as the mean particle diameter.

Total phenolic content measurement

As previously described, the TPC was quantified using the Folin-Ciocalteu method (Sricharoen et al., 2015). Briefly, 2.5 mL of the lignin solution (2 g/L) was mixed with 2.5 mL of Folin-Ciocalteu reagent and 5 mL of a 20% sodium carbonate solution. The resulting mixture was adjusted to a total volume of 50 mL with distilled water and allowed to react at 40°C for 30 min. Subsequently, the absorbance of the solution was measured at 750 nm using ultraviolet-visible (UV-Vis) spectroscopy. The standard curve was based on gallic acid (5–700 mg/L). The results obtained from the UV-Vis spectroscopy were expressed as the percentage of total phenolic content in the produced lignin, represented in terms of gallic acid equivalents.

Antioxidant activity

The antioxidant activity was analyzed based on DPPH assay. Briefly, separate samples of 2.5 mL of lignin solution (1 g/L) were diluted with 5, 25, 50, 125 and 250 mg/L of ethanol. The resulting solutions were mixed with 1 mL of 0.3 mM of DPPH and left to react for 30 min. Then, the absorbance of the final solutions was measured using UV-Vis spectroscopy at 518 nm (Queiroz et al., 2009).

Antimicrobial activity

The antimicrobial activity was analyzed using the ASTM E2149-13a method, according to Aloui et al. (2021), based on the antimicrobial activity of the produced lignin against a representative Gram-positive microorganism (*Staphylococcus aureus*) and a Gram-negative microorganism (*Escherichia coli*).

Statistical analysis

Data were subjected to analysis of variance using the Statgraphics® plus software (version 5.1; Manugistics Inc.; Rockville, MD, USA). Mean comparisons were performed using Fisher's test. Differences between means were considered significant when the confidence interval was smaller than 5% ($p < 0.05$). Results were presented as mean ± SD values.

Results and Discussion

Effect of pretreatment on oil palm empty fruit bunch composition

The components of untreated and dilute sulfuric acid-pretreated OPEFBs at varying acid concentrations (4%, 8% or 10% H_2SO_4) are presented in Table 1, along with corresponding significant differences. The untreated OPEFB consisted of 49.8 ± 0.55% cellulose, 26.0 ± 0.36% hemicellulose and 19.6 ± 0.07% lignin. The data in Table 1 indicated that pretreatment with 8% and 10% H_2SO_4 was significantly more effective in hemicellulose removal compared to 4% H_2SO_4. Ishola et al. (2014) and Khomlaem et al. (2023) established that dilute acid pretreatment was significantly more effective than high-concentration acid pretreatment in hemicellulose solubilization.

Table 1 Oil palm empty fruit bunch composition before and after pretreatment with dilute sulfuric acid

Component	Dry composition (%, weight per weight)			
	Untreated	Pretreated with 4% H_2SO_4	Pretreated with 8% H_2SO_4	Pretreated with 10% H_2SO_4
Cellulose	49.8 ± 0.55^a	49.4 ± 0.01^a	50.4 ± 0.16^a	51.0 ± 0.70^a
Hemicellulose	26.0 ± 0.36^a	23.1 ± 0.05^b	17.1 ± 0.10^c	16.4 ± 0.64^c
Lignin	19.6 ± 0.07^c	26.3 ± 0.08^b	30.9 ± 0.47^a	31.0 ± 0.32^a
Ash	4.64 ± 0.01^a	1.22 ± 0.02^b	1.64 ± 0.01^b	1.60 ± 0.01^b

Mean ± SD values ($n = 3$) in each row superscripted with different lowercase letters are significantly ($p < 0.05$) different.

However, the lignin composition achieved stability at a dilute sulfuric acid concentration of 8%. After the pretreatment, the composition of OPEFB included 50.4 ± 0.16% cellulose, 17.1 ± 0.10% hemicellulose and 30.9 ± 0.47% lignin. The hemicellulose content was 34% less in the OPEFB subjected to the 8% H_2SO_4 pretreatment. Additionally, lignin pretreated with 8% H_2SO_4 had the highest dry weight percentage (1.58 times greater than for the untreated OPEFB), as shown in see Table 1. This highlighted that an 8% sulfuric acid pretreatment was well-suited for further exploration in lignin extraction ($p < 0.05$).

Lignin extraction using organosolv technique

Lignin was extracted from the black liquor obtained using the organosolv technique by applying different mixtures of formic acid, acetic acid and water (30/60/10, 45/45/10 and 60/30/10% (v/v/v)), as well as varying extraction durations (30, 45, 60 or 90 min). The extraction efficiency of lignin was evaluated as a percentage using Equation 1. The purity of the lignin was determined by summing the quantities of acid-insoluble lignin (AIL) and acid-soluble lignin (ASL). All results of the study are depicted in Table 2.

The current investigation showed that the efficiency of lignin extraction decreased as the concentration of formic acid increased, concomitant with a reduction in acetic acid concentration (as detailed in Table 2). Acetic acid is an organic acid that acts as a solvent in lignin extraction and has been commonly utilized in the acetosolv technique, emphasizing its importance in lignin extraction (Hidayati et al., 2017).

However, the inclusion of formic acid has been shown to enhance the efficiency of lignin extraction and yield greater lignin purity compared to the exclusive use of acetic acid due to its greater efficacy in breaking ether bonds (Hidayati et al., 2017). Some investigations have examined the sole use of formic acid for lignin extraction, indicating that both the effectiveness of lignin extraction and lignin purity were lower than those achieved through the exclusive use of acetic acid (El Mansouri and Salvadó, 2006; Ramakoti et al., 2019).

As shown in Table 2, the highest lignin extraction efficiency of 45.0 ± 0.12% was obtained using the mixture of formic acid, acetic acid and water (FA/AA/H_2O) of 30/60/10% (v/v/v) for 90 min. The lignin purity was directly proportional to the efficiency of lignin extraction. The significantly highest purity of 92.4 ± 0.10% was obtained using the same conditions (FA/AA/H_2O of 30/60/10% (v/v/v) for 90 min). The longer the extraction time, the higher the lignin extraction efficiency (Table 2), because hydrogen ions can destroy polymers in the molecular structure of lignin (Ganewatta et al., 2019). However, if the concentration of hydrogen ions in the solution is too high during lignin extraction, rearrangements of carbocation atoms may occur, causing the re-lignin polymerization reaction, which results in the lignin extraction efficiency and lignin purity becoming constant and decreasing, respectively (Hidayati et al., 2017). There were not significant differences in the lignin extraction efficiency and lignin purity between the extraction times of 60 min and 90 min. Therefore, the optimal extraction conditions should be a mixture of FA/AA/H_2O of 30/60/10% (v/v/v) for 60 min. The efficiency of lignin extraction was 44.1 ± 0.06%. Lignin purity was 92.2 ± 0.21%,

Table 2 Efficiency and purity of lignin extraction in various mixtures of organic acid and extraction times

Formic acid/Acetic acid/ Water (% v/v/v)	Extraction time (min)	Efficiency of lignin extraction (%)	AIL (%)	ASL (%)	Lignin purity (%)
30/60/10	30	29.1 ± 0.06^{cw}	85.4 ± 0.59^{cw}	1.11 ± 0.02^{aw}	86.5 ± 0.57^{cw}
	45	35.5 ± 0.07^{bx}	88.6 ± 0.80^{cx}	1.16 ± 0.03^{cx}	89.8 ± 0.39^{cx}
	60	44.1 ± 0.06^{cy}	91.0 ± 0.22^{cy}	1.16 ± 0.02^{ax}	92.2 ± 0.21^{cy}
	90	45.0 ± 0.12^{cy}	91.2 ± 0.11^{cy}	1.15 ± 0.02^{ax}	92.4 ± 0.10^{cy}
45/45/10	30	24.7 ± 0.18^{bw}	81.9 ± 0.50^{bw}	1.25 ± 0.02^{bw}	83.2 ± 0.50^{bw}
	45	28.5 ± 0.12^{ax}	84.4 ± 0.46^{bx}	1.14 ± 0.03^{ax}	85.5 ± 0.49^{bx}
	60	30.8 ± 0.04^{ay}	89.3 ± 0.30^{by}	1.21 ± 0.01^{bx}	90.5 ± 0.29^{by}
	90	30.9 ± 0.14^{ay}	88.6 ± 0.71^{by}	1.21 ± 0.02^{by}	89.8 ± 0.70^{by}
60/30/10	30	23.6 ± 0.20^{aw}	79.8 ± 0.47^{aw}	1.09 ± 0.03^{aw}	80.9 ± 0.47^{aw}
	45	28.8 ± 0.13^{ax}	82.8 ± 0.71^{ax}	1.10 ± 0.02^{aw}	83.9 ± 0.70^{ax}
	60	32.0 ± 0.02^{by}	87.3 ± 1.05^{ay}	1.38 ± 0.04^{cx}	88.7 ± 1.03^{ay}
	90	32.1 ± 0.20^{by}	87.2 ± 0.28^{ay}	1.27 ± 0.01^{cy}	88.5 ± 0.29^{ay}

v/v/v = volume per volume per volume; AIL = acid-insoluble lignin; ASL = acid-soluble lignin; Mean ± SD ($n = 3$) in each column superscripted with different lowercase letters indicate significant ($p < 0.05$) differences for various mixtures of organic acid and extraction times.

containing 91.0 ± 0.22% of acid-insoluble lignin (AIL) and 1.16 ± 0.02% of acid-soluble lignin (ASL). Pramana et al. (2021) performed lignin isolation using the organosolv method and OPEFB and obtained a highest purity of 94.49% for FA/AA/H_2O of 30/60/10% (v/v/v) at 121°C for 4 hr that was comparable to the current data.

In addition, the lignin produced from all extraction conditions in the current study could be applied to cosmetics and packaging products, because its purity was within the acceptable ranges for these applications (Inna et al., 2018; Gordobil et al., 2020). Fig. 2 contains an image of the OPEFB before treatment and after organosolv, as well as lignin precipitation.

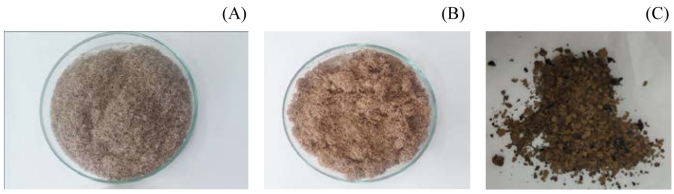

Fig. 2 Samples of materials involved in processing: (A) raw oil palm empty fruit bunches (OPEFBs); (B) OPEFBs after organosolv treatment; (C) lignin after precipitation

Fourier-transform infrared analysis

FTIR analysis was conducted to identify the chemical structure and functional groups of the produced lignin from the OPEFB. The FTIR spectrum of the produced lignin is shown in Fig. 3 and the chemical functional groups at various wavenumbers are shown in Table 3. The results were consistent with Hu et al. (2012), Erdocia et al. (2014) and Medina et al.(2015). Some groups, including syringyl, guaiacyl, p-hydroxyl propane, –OH and various aldehyde group residues, were generally present in lignin.

As seen in Fig. 3, the FTIR spectra in the wavenumber range 400–4000 cm^{-1} indicated that the produced lignin from the OPEFB in the current study were within the absorption band standard range and relevant to general lignin constituent groups, with absorption at wavenumbers of 2922–2842 cm^{-1} for the C–H stretching vibrations of the CH_3 or CH_2 groups, 1714–1704 cm^{-1} for the C=O bond stretching of the carboxyl group, 1602–1594 and 1507 cm^{-1} for the aromatic ring in lignin, 1458-1450 cm^{-1} for the C–H bending of the aromatic hydroxyl group, 1423–1421 cm^{-1} for C–H from lignin transformation, ~1320 cm^{-1} for the syringyl ring linkage with C–O stretching, 1222–1214 cm^{-1} for the C–O stretching of the ester bond in syringyl and guaiacyl, ~1162 cm^{-1} for the ester bond in p-hydroxyphenyl propane, 1119–1111 cm^{-1} for the deformation of aromatics in syringyl and guaiacyl, 1036–1026 cm^{-1} for the C–H and C–O deformation in the guaiacyl structure and 849–845 cm^{-1} for C–H stretch in the 2 and 6 positions of the syringyl structure (Table 3). It was concluded that the produced lignin from the OPEFB was in fact lignin.

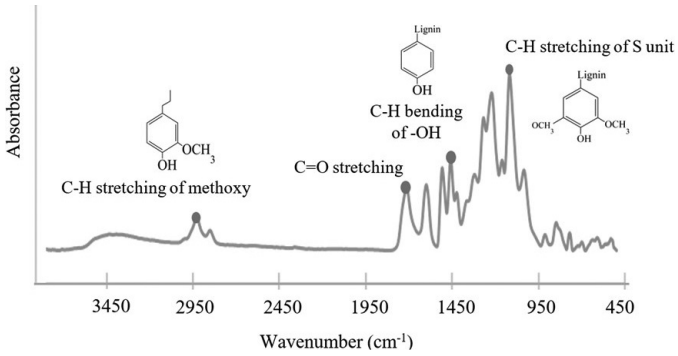

Fig. 3 FTIR spectrum of produced lignin from oil palm empty fruit bunches

Table 3 Fourier-transform infrared spectroscopy functional groups identification for lignin produced from oil palm empty fruit bunches

Absorption band (cm^{-1})	Assignment
2922–2842	C–H stretch in CH_3 or CH_2 groups
1714–1704	C=O stretch in the unconjugated carboxyl
1602–1594 and 1507	aromatic skeletal vibrations and C=O stretch
1458–1450	C–H bending of an aromatic hydroxyl group
1423–1421	aromatic skeletal vibrations combined with C–H in-plane deformation
~1320	syringyl ring breathing and C–O stretching
1222–1214	C–O stretch in the ester of syringyl and guaiacyl
~1162	Stretch of ester bond in p-hydroxyphenyl propane
1119–1111	deformation for aromatics in syringyl and guaiacyl
1036–1026	C–H and C–O deformation in guaiacyl structure
849–845	C–H stretch in 2 and 6 positions of syringyl structure

Gel permeation chromatography analysis

Based on the GPC analysis, the mean lignin weight and mean molecular weight (M_w and M_n) were 2,228 Da and 1,471 Da, respectively, with a polydispersity (M_w/M_n) of 1.5. The molecular weight distribution curve is shown in Fig. 4. Compared to (Lora, 2008), the current technique produced a lower molecular weight of lignin than other extraction techniques, because during the reaction in an acidic environment, the hydrogen ions (H^+) are the leading cause of the depolymerization reaction of macromolecular lignin at the α-aryl and β-aryl ether linkages. Subsequently, it forms carbon cations, connecting to the aromatic rings of other lignin units with many anion electrons. In addition to the mechanism described above, it was confirmed that the produced lignin from OPEFB at 121°C consisted of small particles, due to the reaction occurring at a high temperature (Erdocia et al., 2014; Hu et al., 2014).

Dynamic light scattering measurement

DLS was used to measure the lignin particle size under optimal conditions, where the lignin purity was 92.2 ± 0.21% within a water-based, colloidal lignin suspension. The mean particle size of the lignin produced from the OPEFB using the organosolv technique was 404.6 nm. The particle size distribution (Fig. 5) was consistent with the lignin sizes reported by Iravani and Varma (2020) and Torres et al. (2021) using the same technique. Furthermore, many studies have shown that different lignin extraction techniques yield different lignin particle sizes due to the properties of lignin, such as solubility, molecular weight and functional groups affecting the size of lignin particles (Ganewatta et al., 2019; Hu et al., 2012; Ramakoti et al., 2019). Lignin with a particle size approaching nanoparticles has a high surface area, resulting in high capability and efficiency in both commercial applications of antioxidant and antimicrobial properties (Tang et al., 2020).

Total phenolic content analysis

The TPC of the produced lignin was determined based on using gallic acid as a standard solution expressed as milligrams of gallic acid equivalent (GAE) per gram of lignin. A high temperature (121°C) was used in the current study to increase the diffusion coefficient during lignin extraction, resulting in good solubility of polyphenol (Andres et al., 2020). The TPC of the lignin from the OPEFB using the organosolv technique was 342.98 ± 1.83 mg GAE/g lignin. The produced lignin yielded a TPC similar to the organosolv extraction results reported by Ramakoti et al. (2019). The extraction conditions for the current study were a mixture of FA/AA/H_2O of 30/60/10% (v/v/v) for 60 min. However, formic acid is an organic acid with strong acid properties that can produce hydrogen ions (H^+) by dissociation if there is too much formic acid in the solution (such as for 45/45/10 and 60/30/10% (v/v/v)). The high H^+ content decreases the total phenol content and destroys the aryl-ether bond (aryl-ether bond cleavage) of the lignin until the carbon cations form a new bond with an anion from an aromatic ring of the lignin subunit, producing a polyphenol with reduced hydroxyl groups (Sridach, 2010; Ramakoti et al., 2019).

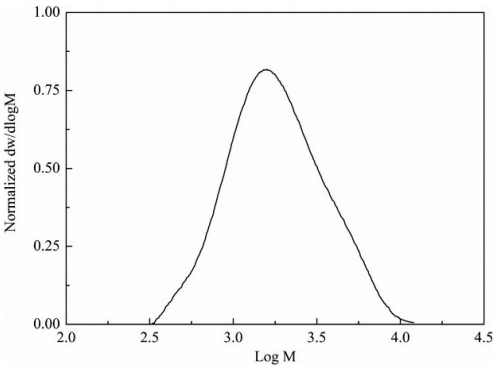

Fig. 4 Gel permeation chromatography molecular weight distribution curve of lignin product (92.2% purity), where w = weight

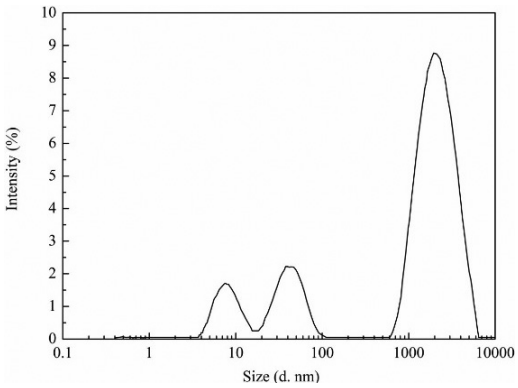

Fig. 5 Scattered light intensity-weighted particle size distribution of lignin (92.2% purity) measured using dynamic light scattering, where d = diameter

Antioxidant activity

The antioxidant activity of lignin produced by the organosolv technique from the OPEFB is shown in Table 4. The significant concentration at which 50% inhibition of free radical activity is observed (IC_{50}) of the produced lignin was 55.24 ± 0.44 µg/mL, which is moderate antioxidant activity compared to common antioxidants and Jadid et al. (2017). In the current study, the lignin produced using the organosolv technique and the OBEFB had a higher antioxidant activity than butylated hydroxytoluene (BHT), by approximately 2.53 times; however, it was still less than for vitamin C and E, because the phenolics in lignin contain hydroxyl and methoxy ions that can oxidize with molecules without electron or free radicals (Erdocia et al., 2014; Hangun-Balkir and McKenney, 2012; Mahmood et al., 2015).

Table 4 Antioxidant activity on produced lignin and other antioxidant agents

Sample	IC_{50} (µg/mL)
Produced lignin	55.24 ± 0.44^{c}
Butylated hydroxytoluene	139.8 ± 1.88^{d}
Vitamin C	4.270 ± 0.10^{a}
Vitamin E	13.88 ± 0.16^{b}

IC_{50} = antioxidant concentration at which 50% inhibition of free radical activity is observed.
Mean ± SD (n = 3) values in a column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Antimicrobial activity

As a result of the antimicrobial activity, 1,000 mg/mL of the produced lignin was cultured individually using *Staphylococcus aureus* and *Escherichia coli* for 1 hr. The results are presented in Table 5. It was found that the produced lignin had a bacterial reduction of 99.6% and an antimicrobial activity of 2.41 with the *S. aureus* culture. However, the produced lignin cultured using *E. coli* had a bacterial decrease of 16.6% and an antimicrobial activity of 0.08. These results indicated that the produced lignin could eliminate both strains within 1 hr and had different levels of antimicrobial activity. The produced lignin could eliminate *S. aureus* more effectively due to the different microbial structural properties of both microorganisms: 1) *S. aureus*, a Gram-positive microorganism, absorbs various substances, including antibiotic agents, more rapidly than *E. coli* (a Gram-negative microorganism); and 2) *E. coli* has an outer membrane layer and more than one cell wall that reduced the antimicrobial ability of produced lignin compared to *S. aureus* (Malanovic and Lohner, 2016). The current results were consistent with Lee et al. (2014), who investigated the antimicrobial activity of lignin nanoparticles tested with *S. aureus* and *E. coli*. Their lignin nanoparticle sample reduced microorganisms of *S. aureus* by 99.9%. However, at the same time, it did not affect the microorganisms of *E. coli* (Lee et al., 2014).

Conclusion

Lignin was extracted from OPEFB samples using organic solvents (formic and acetic acids). The optimal extraction obtained using a mixture of formic acid, acetic acid and water (FA/AA/H_2O; 30% (v/v/v)/60% (v/v/v)/10% (v/v/v) for 60 min was significantly effective for lignin extraction and lignin purity at 44.1 ± 0.06% and 92.2 ± 0.21%, respectively. The produced lignin had a small molecular size and most of the functional groups were phenol hydroxyl groups, which affected the antioxidant and antimicrobial activities. The organosolv technique produced a high TPC (342.98 ± 1.83 mg GAE/g lignin) and had better antioxidant activity than BHT. In addition, the produced lignin could resist *S. aureus* microorganisms (99.6%). Overall, the organosolv technique showed good potential for lignin extraction from OPEFBs to enhance the antioxidant and antimicrobial activities and also as a way to add value to agricultural waste materials.

Table 5 Antimicrobial activity of produced lignin according to ASTM E2149-13a (Aloui et al., 2021) method

Sample	Remark	*Staphylococcus aureus*	*Escherichia coli*
Buffer	Quantities of microbe (CFU/mL)	1.43×10^{5}	3.51×10^{5}
Produced Lignin	Quantities of microbe (CFU/mL)	5.60×10^{2}	2.93×10^{5}
	Antimicrobial activity	2.41	0.08
	Reduction (%)	99.6	16.6

Conflict of Interest

The authors declare that there are no conflicts of interest.

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