DOI: https://doi.org/10.62985/j.huit_ojs.vol26.no3.444

RECOVERY OF LIGNIN FROM COCONUT COIR (*Cocos nucifera* L.) BY REUSABLE DEEP EUTECTIC SOLVENTS: CHARACTERIZATION AND POTENTIAL APPLICATION IN GAUZE FABRIC COATING

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Received: 1 June 2026; Revised: 16 June 2026; Accepted: 22 June 2026

ABSTRACT

This study investigated lignin recovery from coconut coir using a choline chloride–oxalic acid deep eutectic solvent and evaluated the solvent reusability and potential coating application. The results showed that the lignin recovery yield reached 18.70% (w/w) based on the dry weight of biomass when using a solvent system composed of choline chloride (ChCl) and oxalic acid (OA) at a molar ratio of 6:4, at a reaction temperature of 100 °C for 3 hours. The reusability of the DES was also evaluated through ten consecutive extraction cycles and showed that the lignin recovery yield did not change significantly. The recovered lignin exhibited a purity of nearly 90% and showed antioxidant activity with an IC_{50} value of 6.02 µg/mL. In addition, the lignin/DES dispersion system exhibited antibacterial activity against two bacterial strains, including *Staphylococcus aureus* and *Escherichia coli* with inhibition zones diameter of 39 mm and 32 mm, respectively. Furthermore, this study preliminarily demonstrated the potential application of lignin as a coating material for gauze fabric through the lignin/DES dispersion system under conditions of 2 hours of immersion time and lignin/DES dispersion at a concentration of 0.1% (w/w), achieving a weight gain percentage of 53.90% (w/w). The results of this study demonstrate that DES is potentially more sustainable than conventional solvent systems for lignin recovery from coconut coir. Moreover, the findings highlight the potential application of lignin in the development of antibacterial coating materials and other value-added products derived from agricultural residues, in line with the principles of the circular economy and green chemistry.

Keywords: Coconut coir, lignin, deep eutectic solvent, reusability, purity lignin.

1. INTRODUCTION

Coconut (*Cocos nucifera* L.) is a perennial plant species characterized as monoecious and represents the sole species of the genus *Cocos* within the Arecaceae family. It is widely cultivated in tropical coastal lowland regions, with a total global cultivation area estimated at approximately 12 million hectares [1]. Coconut coir is an abundant agricultural by-product containing approximately 35% cellulose, 14.63% hemicellulose, 10.16% ash, and 42% lignin [2], [3], [4]. Due to its abundant lignin content, coconut coir has considerable potential as a feedstock for lignin recovery.

Lignin is a natural polymer that plays an essential role in water and mineral transport, provides mechanical strength for plant development, and helps protect wood against microbial attack [5]. Depending on the source material, the structure and morphology of lignin in nature are very diverse, especially the method of extraction [6]. Therefore, the structure of lignin has not been clearly studied due to its complexity. However, it has been confirmed that in any plant or extraction method, lignin is composed of three main phenylpropane monomers: sinapyl alcohol, coniferyl alcohol, and *p*-coumaryl alcohol [7]. The lignin structure contains various functional groups, including phenolic hydroxyl, carboxyl, benzyl alcohol, and methoxyl groups [8]. Phenolic compounds and carbohydrates are linked through covalent bonds. In addition, the monomeric units within lignin are interconnected via ether linkages (β–O–4, 4–O–5, β–5) and carbon–carbon linkages (β–β, 5–5, β–1). Among these, the β–O–4

linkage accounts for approximately 40 – 60% of the total linkages, while 5–5 linkages contribute about 10 – 20% and β–5 linkages account for approximately 10 – 12% [9]. The phenolic functional groups in lignin also impart strong antioxidant and antibacterial properties, as well as ultraviolet absorption capacity [10]. Therefore, in the packaging, food wrapping, medical materials, and anti-aging cosmetics industries, lignin is used as a carrier material with antioxidant activity to support these industries [11].

Currently, the extraction of natural compounds from agricultural by-products has attracted considerable attention due to their potential applications across various industrial fields. Lignin is a natural polymer with notable biochemical properties, such as antioxidant and antibacterial activities, making it applicable in industries including papermaking, modified adsorbent materials, and antibacterial textiles. However, conventional methods such as alkaline or acid treatments and the use of organic solvents still present significant environmental drawbacks. In this situation, deep eutectic solvents (DESs) have emerged as a new generation of solvents with strong potential for the sustainable and environmentally friendly extraction of lignin from biomass.

Deep eutectic solvent (DESs) systems were first introduced by Abbott et al. in 2004. These solvents are formed from a combination of hydrogen-bond acceptors (HBAs) and hydrogen-bond donors (HBDs), resulting in a system with a lower melting point than that of the individual components and a dynamic, stable hydrogen-bonding network. In addition, their simple synthesis process and the availability of raw materials contribute to positioning DESs as next-generation solvents, offering significant potential to replace conventional ionic solvents in lignocellulosic biomass processing [12].

DES are considered green solvents and are widely used in lignocellulosic biomass processing. Cronin et al. reported that the use of DESs for lignin removal from straw achieved a lignin recovery yield of 75% with a purity of 90% [13]. Another study by Fernandes et al. developed a novel DES system by combining lactic acid (LA), tartaric acid (TA), and choline chloride (ChCl) at a molar ratio of 4:1:1 for lignin extraction from sawdust, achieving a lignin extraction yield of 95% and a lignin purity of 89% under conditions of 175 °C for 1 hour [14]. Numerous studies have demonstrated the effectiveness of DESs in biomass pretreatment and lignin extraction due to their excellent lignin-dissolving capability. However, most previous investigations have primarily focused on maximizing lignin recovery yields or evaluating the properties of the obtained products, whereas the reusability of DESs has not been thoroughly investigated, particularly for coconut coir biomass. This represents a significant limitation, as solvent cost and the ability to maintain extraction performance over multiple reuse cycles are key factors determining both the economic feasibility and industrial applicability of the process. In light of this limitation, the present study investigates the extraction and recovery of lignin from coconut coir using DES, with parameters including the molar ratio of choline chloride (ChCl) to oxalic acid (OA), reaction temperature, and reaction time, as well as the reusability of the solvent over multiple extraction cycles. The selection of the ChCl/OA solvent system in this study was based on experimental evidence from previous studies demonstrating that lignin extracted using a DES composed of choline chloride (ChCl) and oxalic acid (OA) exhibits superior biological properties compared with lignin obtained from systems in which oxalic acid was replaced by lactic acid or monoethanolamine. Specifically, evaluations of antioxidant activity using both DPPH and ABTS assays, as well as the UV-shielding performance of lignin incorporated into PVA films, revealed that lignin extracted with oxalic acid possessed more favorable properties than that extracted using lactic acid or monoethanolamine [15]. Therefore, the ChCl/OA solvent system was selected in the present study to maximize the biological value of lignin recovered from coconut coir.

In addition to evaluating the physicochemical properties of lignin, including its purity, antioxidant activity, and antibacterial activity, this study also explored the potential application of lignin in biomedical materials. Previous studies have reported the deposition of lignin onto cotton substrates, nonwoven fabrics, and polymer films to enhance their mechanical properties as well as their antibacterial and antioxidant performances [16]. Owing to its excellent absorbency, biocompatibility, and widespread use in wound care applications, gauze fabric was selected as the substrate for evaluating the applicability of the lignin/DES system. Therefore, the study further conducted a preliminary investigation of the coating process of the lignin/DES dispersion system onto gauze fabric by examining the effects of immersion time and lignin dispersion concentration, with the aim of identifying suitable conditions for improving lignin deposition efficiency on the gauze surface.

2. MATERIALS AND METHODS

2.1. Materials and chemicals

Coconut husk (*Cocos nucifera L.*) was collected in Tan Phu District, Ho Chi Minh City. After collection, the shell was removed, and the remaining coir was cut into segments of approximately 3 – 4 cm in length. The material was then thoroughly washed to remove impurities and dried at 60 °C until completely dry. The dried coir was ground and sieved through a 500 µm mesh to obtain the experimental sample, which was stored at room temperature.

The chemicals used in this study were of analytical grade, including choline chloride, oxalic acid, acetone, dimethyl sulfoxide, and sulfuric acid (Xilong, China), 2,2-diphenyl-1-picrylhydrazyl and gallic acid (Sigma-Aldrich, America), *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 8739) were stored at the Drug Research and Development Center.

2.2. Research methodology

2.2.1. Extraction and recovery of lignin using deep eutectic solvent (DES)

Preparation of the DES system: ChCl and OA were mixed at an appropriate molar ratio. The amounts of individual components were calculated and adjusted according to the target molar ratio and the desired quantity of DES to be synthesized. The solid mixture was stirred in a water bath at 60 °C on a magnetic stirrer for approximately 30 minutes at a stirring speed of 200 rpm until a homogeneous and clear mixture was obtained, the DES exhibited a pH value of approximately 1. The DES was then cooled and stored at room temperature.

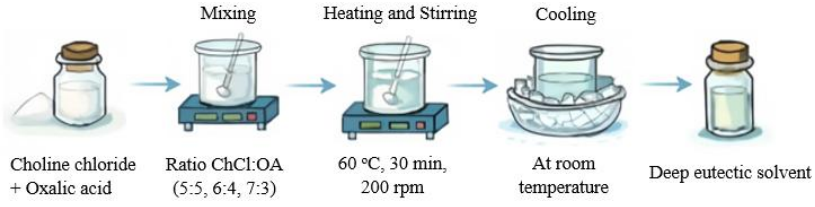

Figure 1. Demonstration of the preparation process of deep eutectic solvents (DES)*
*(*AI-assisted tools were used in the preparation of this figure)*

Lignin extraction and recovery lignin procedure: Approximately 0.5 ± 0.0001 g of coconut coir was weighed into a 50 mL centrifuge tube, followed by the addition of 10 ± 0.0001 g of DES. The tube was tightly capped, and lignin extraction was subsequently carried out by heating in a drying oven. The molar ratios of ChCl:OA investigated were 5:5, 6:4, and 7:3 at temperatures of 80, 90, 100, 110 and 120 °C over extraction times of 1, 2, 3, 4, and 5 hours. After DES treatment, add 10 mL acetone 1:1 to the mixture and centrifuged at 5000 rpm for 10 minutes. The mixture was filtered by Buchner filtration to separate the solid from the filtrate. The residue was washed until the washing solution became colorless, and the washings were combined with the filtrate. Distilled water was added to the filtrate at a volume three times that of the filtrate to induce lignin precipitation, and the mixture was left to stand for 45 minutes. The lignin was further washed with distilled water and centrifuged repeatedly until the washing solution became colorless. Finally, the lignin was dried at approximately 90 °C to obtain lignin powder, and its mass was measured to determine the lignin recovery yield. The lignin recovery yield on a biomass basis was calculated according to Equation (2.1).

$$Lignin\ yield\ (\%) = \frac{mass\ of\ recovered\ lignin}{mass\ of\ biomass} \times 100 \quad (2.1)$$

2.2.2. Reusability of deep eutectic solvent (DES)

The procedure was carried out similarly to that described in section 2.2.1 with parameters such as the ChCl:OA molar ratio, temperature, and reaction time fixed at the best conditions determined from the previous experiments. After centrifugation to recover lignin, the DES phase was subjected to rotary vacuum evaporation at 90 °C and approximately 200 mbar to remove water until the final mass was equal to the initial mass of the DES employed in the extraction process, thereby obtaining DES none water. The recovered DES was then reused for subsequent lignin extraction cycles. This process was

repeated for 10 cycles and the variation in lignin recovery yield of the recycled DES was recorded.

2.2.3. Evaluation of lignin properties

Purity: Lignin was hydrolyzed in concentrated acid (H_2SO_4, 72%). The acid-soluble lignin (ASL) fraction was determined using a spectrophotometric method (TAPPI-UM 250) [17], the acid-insolube lignin (AIL fraction was determined by the gravimetric method (TAPPI T 222 om-02) [18].

Approximately 0.1 ± 0.0001 g of lignin sample was accurately weighed into a glass beaker, and the sample mass was recorded (m_m). Then, 3 mL H_2SO_4 72% was added, and the mixture was gently stirred to ensure complete homogenization. The mixture was maintained at 30 °C for 60 minutes with gentle stirring every 5 minutes. Subsequently, 84 mL of distilled water was added to dilute the acid to a concentration of 4%, and the solution was thoroughly mixed to ensure homogeneity. The mixture was then filtered through a pre-weighed filter paper (m_2) using Buchner filtration. The filter paper containing lignin was washed until pH 7 (the washings were combined with the filtrate, but the total volume did not exceed 100 mL). The filter paper with lignin residue was dried at 90 °C to constant weight, and the final mass was recorded (m_1). The percentage of acid-insoluble lignin (AIL (%)) was calculated according to Equation (2.2).

$$\text{AIL}\ (\%) = \frac{m_1 - m_2}{m_m} \times 100 \qquad (2.2)$$

Where m_1 is the mass of the filter paper and acid – insoluble lignin (g), m_2 is the mass of the filter paper (g), and m_m is the mass of the lignin sample (g).

The filtrate (combined with the washings) was diluted to a final volume of 200 mL using H_2SO_4 4%. The absorbance was then measured over the wavelength range of 200 – 400 nm. The percentage of acid-soluble lignin (ASL (%)) was calculated according to Equation (2.3).

$$\text{ASL}\ (\%) = \frac{\text{Abs} \times V}{\varepsilon \times m_m \times b} \times f \times 100 \qquad (2.3)$$

Where Abs is absorbance at wavelength 280 nm, V (mL) is a final volume, ε is the molar absorptivity coefficient, with a value of 9,447 (L/g.cm), mm is the mass of lignin sample (mg), b is cuvette path length, with a value of 1 cm, and f is the dilution factor [19].

Purity of lignin was calculated according to Equation (2.4).

$$P\ (\%) = \text{ASL}\ (\%) + \text{AIL}\ (\%) \qquad (2.4)$$

Antioxidant activity: The antioxidant activity of lignin was evaluated based on its free radical scavenging ability against DPPH, following the method developed by Ruiz-Torralba [10]. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical commonly used for screening the antioxidant activity of studied compounds. The antioxidant activity is indicated by the reduction in DPPH color, which is determined by measuring the absorbance at 517 nm. Gallic acid (GA) was used as the positive control.

The DPPH solution was prepared and adjusted to obtain an absorbance of approximately 0.7 at 517 nm. The experiment was conducted in triplicate, and the absorbance of the control sample (Ac) was recorded. Approximately 0.01 ± 0.0001 g of lignin sample was accurately weighed into a glass beaker, followed by the addition of 3 mL of DMSO to completely dissolve the sample. The solution was then diluted to a volume of 10 mL with DMSO to obtain a stock solution with a concentration of 1000 µg/mL. The stock solution was further diluted with DMSO to prepare a working solution at a concentration of 100 µg/mL. Calibration curves (y = ax + b) were constructed using lignin concentrations of 2, 4, 6, 8, and 10 µg/mL, and gallic acid (GA) concentrations of 0.4, 0.8, 1.2, 1.6, and 2.0 µg/mL. Each experimental point was prepared in ethanol to a total volume of 5 mL.

The percentage of DPPH inhibition (% I) for the lignin sample and gallic acid was calculated according to Equation (2.5).

$$\%I = \frac{A_C - A}{A_C} \times 100 \qquad (2.5)$$

Where A_C is absorbance of control sample and A is absorbance of lignin sample or gallic acid.

Antibacterial activity by disk diffusion method: The antibacterial activity of the samples was determined using the agar well diffusion method as described in [20]. The microbial inoculum was prepared by diluting the liquid culture to achieve a turbidity equivalent to the 0.5 McFarland standard, followed by further dilution to obtain a cell density of 1.5×10^8 CFU/mL. A volume of 100 µL of the

microbial suspension was uniformly spread onto the surface of Petri dishes containing MHA. Subsequently, wells with a diameter of 7 mm were created using a sterile cork borer.

A volume of 50 µL of lignin dispersed in DES (ChCl:OA, molar ratio 6:4) at a dispersion concentration of 2000 µg/g was added into each well. Gentamicin 25 µg/mL was used as the positive control, while DES was used as the negative control in the antibacterial assay. The agar plates were incubated for 24 hours, after which the results were observed and recorded. The antimicrobial activity was evaluated by measuring the diameter (D) of the inhibition zone according to Equation (2.6).

$$\mathrm{DK\ (mm)} = \mathrm{D} - \mathrm{d} \quad (2.6)$$

Where D (mm) is the diameter of the inhibition zone and d (mm) is the diameter of the agar well (7 mm).

Surface morphology analysis by field emission scanning electron microscopy (FE-SEM): FE-SEM measurements were carried out using a Hitachi S-4800 system (Hitachi High-Tech, Japan). The sample was sputter-coated with platinum (Pt) to improve electrical conductivity, a coating thickness of 5 nm was deposited at a sputtering current of 20 mA for 60 seconds. Images were recorded in secondary electron mode (SE(M)) at an accelerating voltage of 10 kV, a working distance of 8.1 mm, and an emission current of 9800 nA. The system was operated in Normal lens mode with the condenser 1 setting at 5000. Samples were observed at a tilt angle of 0^o and a rotation angle of 0^o.

Chemical composition analysis by FTIR spectroscopy: Fourier transform infrared (FTIR) spectroscopy was performed using an FT/IR-6X spectrometer (Jasco, Japan) to identify characteristic vibrational bands corresponding to chemical bonds in lignin extracted from coconut coir. The sample was placed directly on the ATR crystal, spectra were recorded over the wavenumber range of 400 – 4000 cm^{-1} with a spectral resolution of 2 cm^{-1}, using the ATR mode. The measurements were conducted using a Jasco FTIR-6X instrument at the International Analysis Center, Ho Chi Minh City University of Industry and Trade.

2.2.4. Coating process of lignin/DES dispersion onto gauze fabric

The gauze used in this study was a medical-grade gauze composed of 100% cotton. Pre-weighed gauze fabrics (m_1) with dimensions of approximately 3×4 cm were used for coating. The lignin/DES system was applied onto the gauze fabric under various immersion times of 1, 2, 3, 4 and 5 hours at different dispersion concentrations of 0.05; 0.1; 0.15; 0.2; 0.25% (w/w). After treatment, the gauze was squeezed to remove excess DES and then rinsed by stirring in 50 mL of distilled water on a magnetic stirrer for 10 seconds at 200 rpm to reduce residual DES on the fabric surface. The fabric was subsequently squeezed to remove excess water and dried at 70 °C until completely dry. The final mass was recorded (m_2) to evaluate the coating efficiency of lignin on the gauze. The deposition efficiency of the lignin/DES system onto gauze fabric was evaluated based on the weight gain (%WG) before and after coating, as calculated using Equation (2.7).

$$\%WG = \frac{m_2 - m_1}{m_1} \times 100 \quad (2.7)$$

Where m_1 (g) is mass of fabric before coating and m_2 (g) is mass of fabric after coating.

The coated gauze was further analyzed by FTIR spectroscopy to confirm the presence and loading efficiency of lignin on the gauze fabric surface.

2.3. Statistical analysis

The experimental data obtained in this study were repeated 3 times. The data were processed and statistically analyzed using one-way analysis of variance (one-way ANOVA) followed by Bonferroni's post-hoc test with a significance level of $\alpha = 0.05$ to evaluate the significance of differences among experimental groups.

3. RESULTS AND DISCUSSION

3.1. Moisture content of material

The coconut coir sample used in the experiment had a particle size of 500 µm and its moisture content was determined to be 7.67 ± 0.06%. This value is below 13%, which falls within the permissible limit for safe storage moisture content according to the Vietnamese Pharmacopoeia IV, thereby

preventing microbial growth and mold formation.

3.2. Effect of processing parameters on lignin extraction using deep eutectic solvent (DES)

Experimental observations indicated that the DES system formed from ChCl and OA achieved a homogeneous and transparent state only when the molar ratio of ChCl was greater than or equal to that of OA. When the DES composition was adjusted to ChCl: OA molar ratios of 4:6 and 3:7, corresponding to a decrease in ChCl content and an increase in OA content, only partial dissolution of OA was observed. Conversely, excessively high ChCl content also prevented the formation of a homogeneous and transparent mixture due to saturation of ChCl, as observed at molar ratios of 8:2 and 9:1. Therefore, this study focused only on investigating ChCl:OA molar ratios of 5:5, 6:4, and 7:3 under fixed reaction conditions (reaction temperature of 90 °C and reaction time of 3 hours).

The evaluation of the effect of the ChCl:OA molar ratio in the DES system showed that increasing the ChCl:OA ratio from 5:5 to 6:4 resulted in an increase in lignin recovery yield from 12.36 (± 0.13)% (w/w) to 14.36 (± 0.21)% (w/w), as presented in Figure 2a. This finding suggests that the extraction efficiency is more strongly influenced by the proportion of ChCl than by OA in the DES system. Depending on the nature of the solvent system employed, different interaction mechanisms may occur between the solvent and biomass structures. Several solvent systems and their corresponding experimental conditions are summarized in Table 1 to provide an overview of lignin recovery yields reported in the literature.

Table 1. Lignin recovery yields obtained using different solvent systems

Solvents	Experimental conditions	Lignin yield (%)	Ref.
DESs	Rice hulls biomass, ChCl:lactic acid (1:10) at 80 ^{o}C in oil bath, 24 hours	19.4	[21]
Sodium hydroxide	OPEFB biomass, NaOH 4% at 80 ^{o}C, 9 hours	Delignification	[22]
Sulfuric acid	Rice hulls biomass, H_2SO_4 2% at 122 ^{o}C	12.9	[23], [24]
Ethanol	Miscanthus biomass, 80% EtOH (H_2SO_4 1%)	Delignification	[25]

The mechanism of lignin extraction using deep eutectic solvents (DESs) is primarily based on the disruption of the hydrogen-bonding network among lignin, cellulose, and hemicellulose. In the ChCl/OA system, chloride ions (Cl^-) from choline chloride compete with the native hydrogen bonds within the biomass matrix, resulting in swelling of the cell wall structure and enhanced solvent penetration. Simultaneously, protons (H^+) released from oxalic acid catalyze the cleavage of β–O–4 ether linkages, which are the predominant linkages in the lignin structure. The resulting lignin fragments are subsequently stabilized through interactions with the DES and dissolved into the solvent phase, thereby facilitating the separation of lignin from the lignocellulosic matrix [26], [27], [28]. Therefore, further increasing the ChCl: OA molar ratio, corresponding to higher choline chloride content and lower oxalic acid content, reduced the lignin recovery yield to 12.41 ± 0.28% (w/w). This can be explained by the decrease in oxalic acid concentration, which increased the system pH and weakened its acidity. Consequently, the protonation process and the cleavage of ether and ester linkages within the lignocellulosic biomass became less effective compared with the ChCl:OA ratio of 6:4, leading to a reduction in lignin extraction efficiency [29].

The effect of temperature was also investigated under fixed conditions of a ChCl:OA molar ratio of 6:4 and a reaction time of 3 hours, as presented in Figure 2b. The lignin recovery yield at 80 ^{o}C was only 10.80 ± 0.25% (w/w), which increased to 14.36 ± 0.21% (w/w) at 90 ^{o}C. The highest lignin recovery yield, 18.28 ± 0.49% (w/w), was obtained at 100 ^{o}C. Under the same ChCl: OA molar ratio, increasing the reaction temperature can enhance the intrinsic properties of DES, such as its ionic characteristics and polarity. This promotes the disruption of intramolecular hydrogen bonds and improves the solubilization of lignin [30]. In addition, increasing the temperature from 80 °C to 100 °C decreases the viscosity of the DES system due to enhanced molecular mobility induced by thermal energy. This facilitates solvent diffusion and penetration into the biomass structure, resulting in more efficient cleavage of linkages within the lignocellulosic matrix. However, when the temperature was further increased to 110 ^{o}C and 120 ^{o}C, the lignin recovery yield decreased significantly to 16.98 ±

0.96% (w/w) and 9.97 ± 0.67% (w/w), respectively. This reduction can be attributed to the degradation of DES components at elevated temperatures, leading to the loss of functional groups responsible for interactions with lignin. Moreover, high-temperature conditions may also promote the formation of cross-linking reactions between carboxylic acids and biomass components [31].

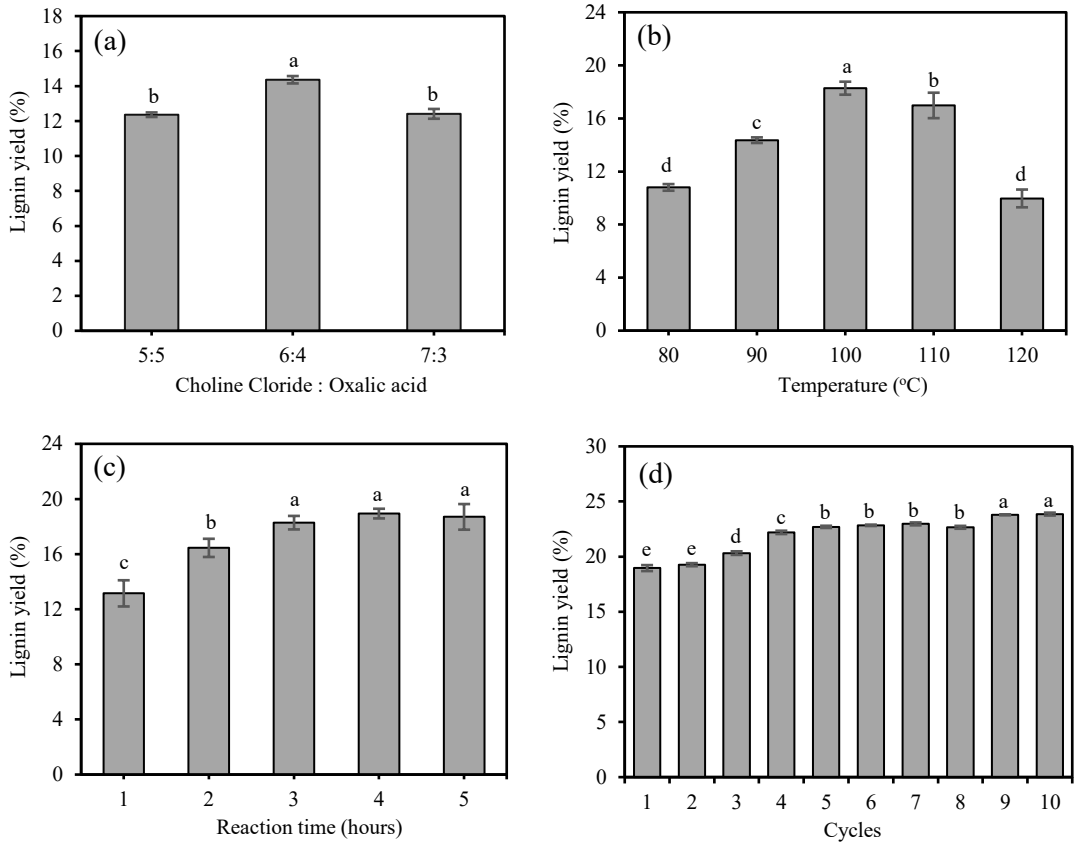

Figure 2. Effect of molar ChCl:OA ratio (a), temperature (b), reaction time on lignin recovery yield (c), and reusability of DES over 10 extraction cycles (d)

*Data are expressed as mean ± SD (n = 3), bars sharing the same letter are not significantly different, whereas bars with different letters indicate significant differences ($p < 0.05$), as determined by one-way ANOVA followed by Bonferroni's post-hoc test

The effect of reaction time on lignin extraction under fixed conditions (ChCl:OA molar ratio of 6:4 and reaction temperature of 100 °C) is presented in Figure 2c. The lignin recovery yield at a reaction time of 1 hour was only 13.15 ± 0.95% (w/w), which increased to 16.45 ± 0.66% (w/w) after 2 hours. This increase can be attributed to the prolonged contact time, which allowed the DES to penetrate and interact more effectively with lignin within the lignocellulosic structure. When the reaction time was further extended to 3 hours, the lignin recovery yield reached a maximum value of 18.28 ± 0.49% (w/w), with no statistically significant differences (p-value > 0.05) compared to the yields obtained at 4 hours (18.94 ± 0.35% (w/w)) and 5 hours (18.70 ± 0.93% (w/w)).

The deep eutectic solvent (DES) was recovered after each extraction cycle and reused for subsequent cycles. The evaluation of DES reusability over 10 lignin extraction cycles is presented in Figure 2d. The results showed that the lignin recovery yield gradually increased from the first to the tenth cycle, rising from 18.96% (w/w) in the first cycle to 23.86% (w/w) in the tenth cycle. This trend may be attributed to the accumulation of residual lignin remaining in the recycled DES system, resulting in an apparent increase in extraction yield over ten repeated cycles. This observation is consistent with previous studies evaluating lignin recovery over four cycles, where the yield increased from approximately 20% in the first cycle to around 30% in the fourth cycle [32]. To provide an objective evaluation of lignin recovery efficiency in this study, the obtained results were compared with previous

studies on lignin extraction from various biomass sources using different treatment methods, as summarized in Table 2.

Table 2. Lignin recovery yield from various biomass sources

Biomass	Treatment method	Lignin yield (%)	Ref.
Loblolly pine	Ethanol (cat: 1.1% H_2SO_4)	18.6	[33]
Wheat straw	Acetic acid (cat: 4% H_2SO_4)	15	[34]
Corn stover	Ionic liquid	30	[35]
Sugarcane bagasse	Alkaline ($Ca(OH)_2$ 1% (w/v))	30	[36]
Fresh bark from larch	Choline chloride/acetic acid + microwave	53	[37]

The comparative results indicate that the lignin recovery yield obtained in this study is comparable to that reported in many previous publications, thereby confirming the feasibility of the proposed process for lignin extraction from coconut coir. Differences in extraction efficiency among studies may be attributed to variations in biomass chemical composition, lignin structure, as well as the treatment conditions and extraction methods employed. Although some methods have achieved higher lignin recovery yields, these processes are often associated with limitations related to environmental impacts. In contrast, deep eutectic solvent (DES) systems, while exhibiting lower extraction efficiency in certain cases, offer significant advantages in terms of reusability, thereby improving lignin recovery sustainability and minimizing environmental concerns. Overall, the reusability of deep eutectic solvents remains feasible and relatively stable across multiple cycles, contributing to improved economic efficiency and sustainability of the process.

3.4. Evaluation of lignin properties

3.4.1. Purity lignin

The purity of lignin is calculated based on the acid-soluble lignin (ASL (%)) and acid-insoluble lignin (AIL (%)) contents for the first five reuse cycles of the DES, these cycles were chosen to represent the lignin recovered over the 10 reuse cycles because no reduction in lignin recovery yield was observed across successive DES reuse cycles. The results are presented in Table 3, and the molecular absorption spectra of lignin are shown in Figure 3.

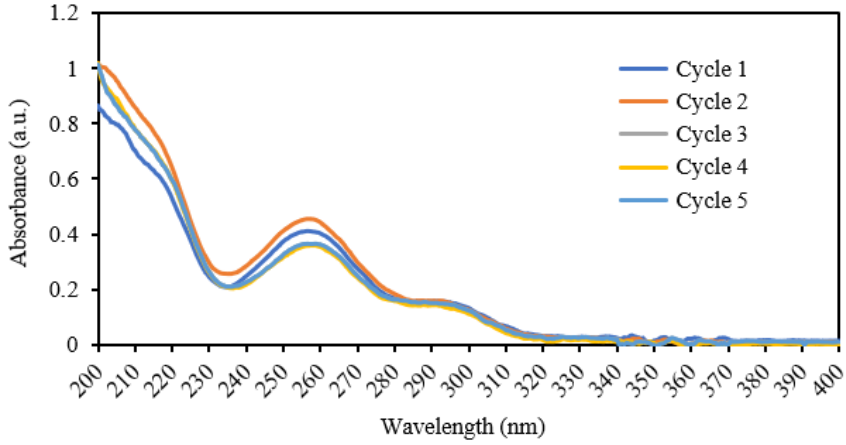

Figure 3. Molecular absorption spectrum of lignin in the wavelength range of 200 – 400 nm

According to previous studies, lignin typically exhibits a maximum absorption peak around 280 nm, attributed to the presence of aromatic structures and conjugated systems within the molecule. However, in this study, the maximum absorption peak of lignin was observed at 260 nm. This hypsochromic shift toward a shorter wavelength may be associated with differences in molecular structure or specific functional groups present in lignin extracted from coconut coir, which can alter its optical absorption characteristics. This result is consistent with previous reports on the UV absorption behavior of lignin, where a similar shift in the absorption maximum was observed [19].

The lignin purity after five extraction cycles showed no statistically significant differences (p-value > 0.05) with values ranging from 73.16% (w/w) to 86.61% (w/w), as presented in Figure 4. This indicates that the use and reuse of DES are highly effective for the extraction and recovery of lignin from coconut coir, achieving a purity close to 90%, which is consistent with previously reported studies [13], [29].

Table 3. ASL, AIL and purity lignin

Criteria	Cycles				
	1	2	3	4	5
Sample mass (mg)	100	101.9	100.2	100.1	101.3
AIL (%)	65.29 ± 5.12^{b}	78.18 ± 4.06^{a}	74.56 ± 4.13^{ab}	75.39 ± 4.79^{a}	75.47 ± 4.70^{a}
ASL (%)	7.87 ± 0.58^{a}	8.43 ± 0.81^{a}	7.54 ± 0.11^{a}	7.87 ± 0.57^{a}	8.09 ± 0.95^{a}
Purity (%)	73.16 ± 4.70^{b}	86.61 ± 4.00^{a}	82.10 ± 4.24^{ab}	83.27 ± 4.47^{ab}	83.56 ± 4.22^{a}

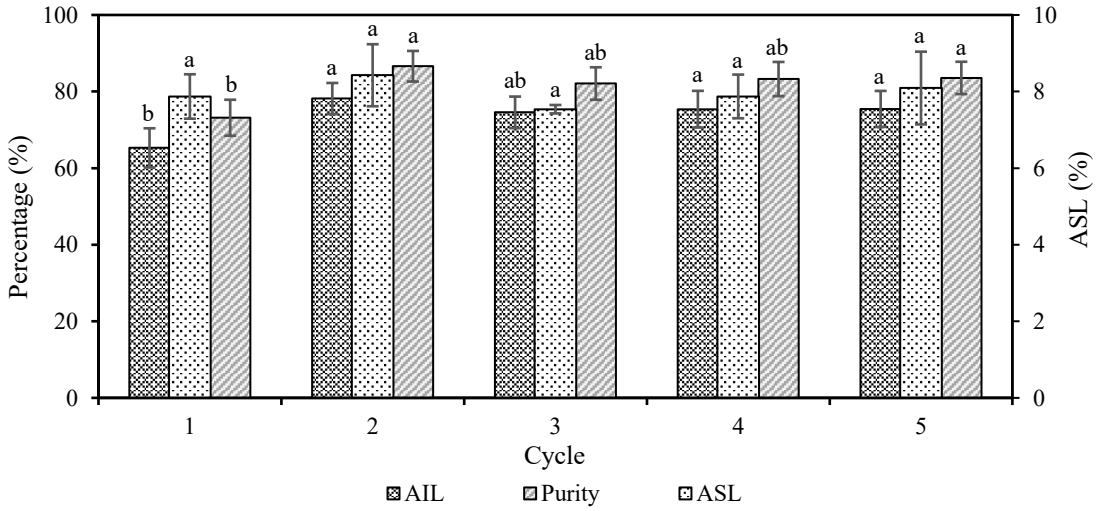

Figure 4. Column chart of ASL, AIL and Purity lignin

*Data are expressed as mean ± SD (n = 3), bars sharing the same letter are not significantly different, whereas bars with different letters indicate significant differences ($p < 0.05$), as determined by one-way ANOVA followed by Bonferroni's post-hoc test

3.4.2. Antioxidant activity using DPPH assay

The absorbance of the gallic acid (GA) standard was measured over a concentration range of 0,4 to 2 µg/mL. The measured absorbance values were converted into the percentage of free radical inhibition (%I) and a linear calibration curve (y = ax + b) was constructed to describe the relationship between GA concentration and DPPH radical inhibition (%I).

Similarly to the GA standard, the absorbance of the lignin sample was measured over a concentration range of 2 to 10 µg/mL. Based on the absorbance values, a linear calibration curve (y = ax + b) was constructed to describe the relationship between lignin concentration and DPPH radical inhibition (%I).

The calibration curve for gallic acid (GA) followed the regression equation y = 40.05x + 2.122 with a correlation coefficient of $R^2 = 0.994$, while the lignin calibration curve followed the regression equation y = 5.227x + 18.513 with a correlation coefficient of $R^2 = 0.9959$, as shown in Figure 5.

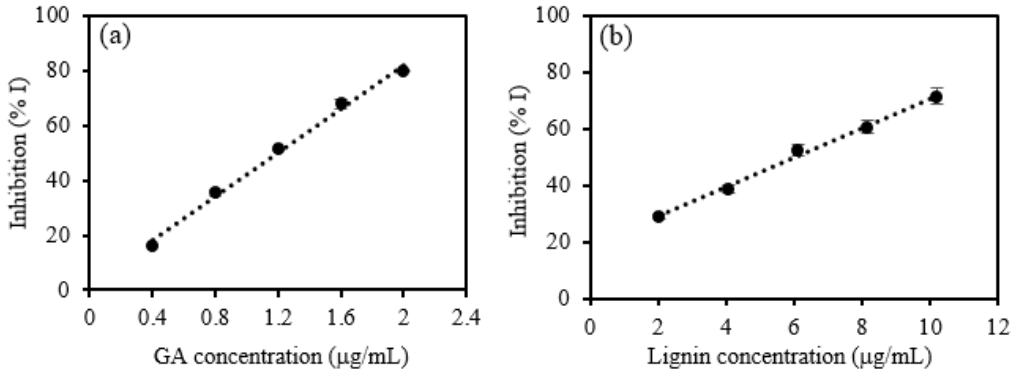

Figure 5. Calibration curves of gallic acid (a) and lignin (b)

The IC_{50} values of GA and the lignin sample were determined based on their respective calibration curves. The results showed that lignin exhibited antioxidant activity with an IC_{50} value of 6.02 µg/mL, which is approximately six times lower than that of the positive control, GA (IC_{50} = 1.19 µg/mL). Although lower than the standard, this result still demonstrated that lignin possessed strong antioxidant activity, indicating its potential for applications in various scientific and material fields.

3.4.3. Antibacterial activity by disk diffusion method

The antibacterial activity results obtained by the disk diffusion method for DES (negative control), gentamicin 25 µg/mL (positive control) and the lignin/DES sample at 2000 µg/g are presented in Figure 6 for two bacterial strains *Escherichia coli* and *Staphylococcus aureus*.

Escherichia coli *Staphylococcus aureus*

Figure 6. Inhibition zones of DES, getamicin and lignin/DES sample

The experimental results indicate that the DES system exhibits notable antibacterial activity. Specifically, the inhibition zone diameters were recorded as 18.5 mm for *Staphylococcus aureus* and 17 mm for *Escherichia coli*. In comparison, the positive control, gentamicin (25 µg/mL), showed inhibition zone diameters of 26 mm and 24 mm against *Staphylococcus aureus* and *Escherichia coli*, respectively. Although the antibacterial efficacy of DES was lower than that of gentamicin, these results still demonstrate its potential as an alternative to conventional antibiotics, particularly in addressing the issue of bacterial resistance [38].

When lignin was dispersed in DES, the diameter of the bacterial inhibition zones increased significantly compared to DES alone. The recorded inhibition zone diameters were 32 mm for *Escherichia coli* and 39 mm for *Staphylococcus aureus*. These findings provide a preliminary evaluation of the antibacterial potential of the lignin/DES dispersion system.

These results indicate that not only DES but also the recovered lignin exhibits strong antibacterial activity. The combination of lignin and DES produces a synergistic effect, enhancing the overall antibacterial performance. Therefore, the lignin/DES dispersion system demonstrates strong feasibility for application in coating gauze fabrics to improve the antibacterial properties of biomedical materials.

3.4.4. Field emission scanning electron microscopy (FE-SEM) analysis result

The surface morphology of lignin was examined using field emission scanning electron microscopy (FE-SEM), and the results are presented in Figure 7. Observations at different

magnifications revealed that lignin exhibits a typical aggregated morphology. At a magnification of 10,000×, the lignin sample appeared as non-uniform particle clusters with sizes in the micrometer range, randomly distributed and forming agglomerated structures.

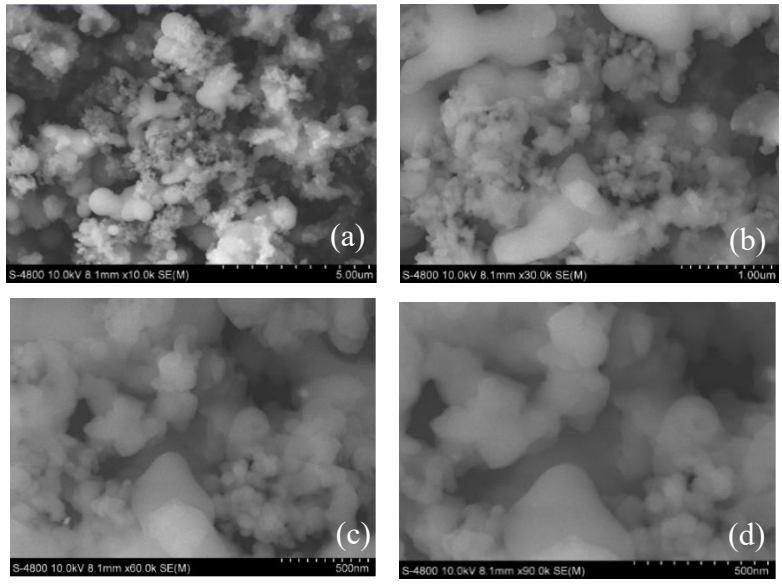

Figure 7. FE-SEM images of lignin at different magnifications ×10,000 (a), ×30,000 (b), ×60,000 (c), and ×90,000 (d)

At higher magnifications, these aggregates were observed to consist of smaller particles with spherical or near-spherical shapes. Based on the scale bars of the FE-SEM images, the size of individual lignin particles was estimated to range from 50 to 200 nm, while the agglomerated clusters could reach sizes of several micrometers. These observations reflect the natural tendency of lignin particles to aggregate due to interactions among aromatic structures and surface functional groups.

3.4.5. Fourier transform infrared (FTIR) analysis result of lignin

The results of the structural characterization of lignin samples are presented in Figure 8. The spectra show that the overall spectral profile of the lignin samples is relatively consistent with typical lignin spectra reported in previous studies [39][40].

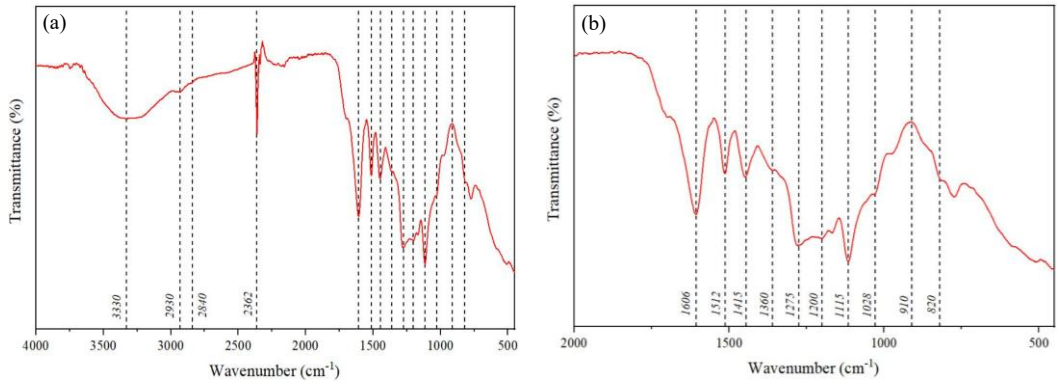

Figure 8. FTIR spectra of lignin in the ranges 4000 – 500 cm^{-1} (a), and 2000 – 500 cm^{-1} (b)

A broad band observed around 3330 cm^{-1} is attributed to the stretching vibrations of hydroxyl (–OH) groups in both phenolic and aliphatic structures of lignin. Additionally, the asymmetric and symmetric stretching vibrations of C–H bonds in methyl and methylene groups are represented by bands at approximately 2930 cm^{-1} and 2840 cm^{-1}. Characteristic vibrations of the aromatic framework are observed at 1606, 1512, 1445 cm^{-1}, indicating that the benzene ring structure of lignin was not degraded

during extraction using DES. Furthermore, the spectra exhibit absorption bands with varying intensities corresponding to guaiacyl (G) units at 1028, 1200 and 1275 cm^{-1}; syringyl (S) units at 1360 and 1115 cm^{-1}; and p-hydroxyphenyl (H) units at 1115 cm^{-1} within the lignin structure. In the lower wavenumber region, bands around 820 cm^{-1}, along with a peak at 910 cm^{-1} are attributed to out-of-plane C–H deformation vibrations at positions 2,6 and 2,5,6 on the aromatic ring. These characteristic vibrations confirm the presence of typical structural units in lignin [15].

3.5. Effect of processing parameters on dispersion lignin onto gauze fabric

The effect of immersion time on the deposition of lignin onto gauze fabric was evaluated based on the weight difference of the gauze samples before and after coating, while maintaining the lignin/DES concentration at a fixed value of 0.2% (w/w), as shown in Figure 9a. When the immersion time increased from 1 hour to 2 hours, the weight gain of the fabric increased from 26.31 ± 6.37% (w/w) to 30.98 ± 5.94% (w/w), indicating that prolonged contact time enhances the penetration and adhesion of the lignin dispersion onto the fabric fibers.

However, when the immersion time was further extended to 3, 4 and 5 hours, the weight gain of the gauze showed no statistically significant difference (p-value > 0.05) compared to the 2-hour sample, with values ranging from 29.25 ± 6.37 to 29.53 ± 4.12% (w/w). This result indicated that, beyond a certain duration, the fabric surface reached a saturation state. Therefore, an immersion time of 2 hours was selected for subsequent experiments.

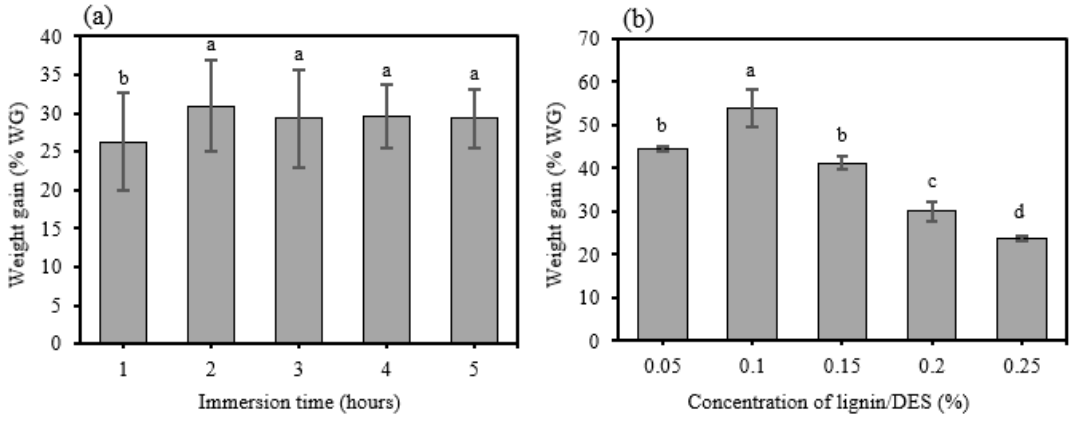

Figure 9. Effect of immersion time (a) and lignin/DES concentration (b) on the deposition efficiency of lignin onto gauze fabric

*Data are expressed as mean ± SD (n = 3), bars sharing the same letter are not significantly different, whereas bars with different letters indicate significant differences ($p < 0.05$), as determined by one-way ANOVA followed by Bonferroni's post-hoc test

The effect of lignin/DES concentration on weight gain is presented in Figure 9b, indicate that at a fixed immersion time of 2 hours, increasing the lignin/DES concentration from 0.05 to 0.1% (w/w) led to an increase in%WG from 44.61 ± 0.54% to 53.90 ± 2.37% (w/w). This suggests that the amount of lignin dispersed in the DES was sufficient to effectively diffuse and adhere to the fabric surface. However, further increasing the lignin concentration in DES to 0.15, 0.2, and 0.25% (w/w) resulted in a gradual decrease in weight gain, with values of 41.11 ± 1.52; 30.02 ± 2.18 and 23.91 ± 0.56% (w/w), respectively. The high standard deviations observed in the immersion time study may be due to the heterogeneous adsorption and retention of the lignin/DES system on the gauze surface among replicate samples.

This phenomenon can be explained based on the dispersion capability and viscosity of the system. Specifically, lignin is effectively dispersed and dissolved in DES due to interactions between lignin and the solvent. At concentrations of 0.05 and 0.1% (w/w), the dispersion system exhibits relatively low viscosity, which facilitates the diffusion and adhesion of lignin onto the gauze surface. Moreover, the higher lignin content in the 0.1% (w/w) system compared to 0.05% (w/w) enhances its retention on the fabric surface, resulting in the highest %WG value at 0.1% (w/w). However, when the dispersion concentration was further increased to 0.15, 0.2, and 0.25% (w/w), the viscosity of the system increased, leading to a reduction in the dispersion efficiency of lignin in DES. At higher concentrations, lignin

tends to aggregate due to attractive interactions between lignin particles in the DES system [41]. Previous studies have also shown that when lignin is well dispersed at an appropriate concentration, the particles can be uniformly distributed on the cellulose surface. In contrast, lignin aggregation results in a non-uniform coating layer and reduces adhesion efficiency on the material surface [42].

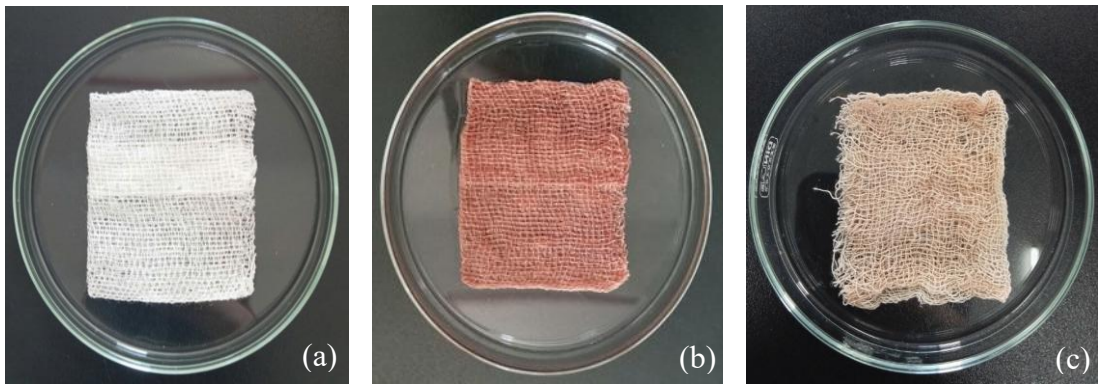

Figure 10. Gauze fabric before coating (a), during coating (b), and after coating (c) with lignin/DES dispersion system at a concentration of 0.1% (w/w)

3.5.3. Fourier transform infrared (FTIR) analysis result of gauze fabric

To evaluate the effectiveness of lignin/DES deposition onto gauze fabric, the lignin-coated sample (coated), prepared under conditions of 2 hours immersion time and a lignin/DES concentration of 0.1% (w/w), along with the uncoated sample (uncoated), were analyzed using Fourier transform infrared (FTIR) spectroscopy. The results are presented in Figure 11.

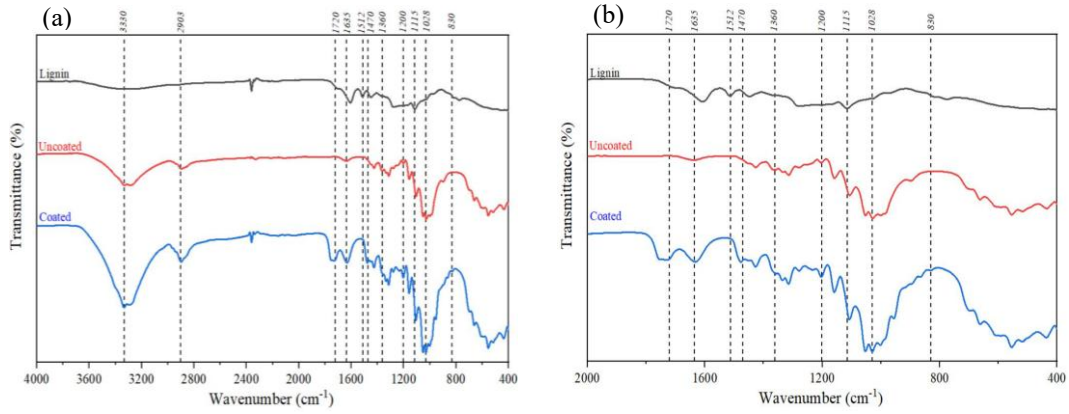

Figure 11. FTIR spectra of uncoated and coated gauze fabric in the ranges 4000 – 400 cm^{-1} (a) and 2000 – 400 cm^{-1} (b)

The FTIR spectra revealed that the uncoated gauze exhibited a broad absorption band around 3330 cm^{-1}, which is characteristic of the stretching vibration of hydroxyl (–OH) groups in cellulose, the primary component of gauze fibers. In the lignin-coated sample, the intensity of the hydroxyl signal increased significantly, which can be attributed to the additional –OH vibrations from phenolic and aliphatic functional groups in lignin deposited on the fiber surface. Furthermore, characteristic lignin peaks were observed, including the asymmetric and symmetric stretching vibrations of hydrogen bonds in methyl and methylene groups within the spectral range of approximately 2800 – 2900 cm^{-1}. Aromatic ring vibrations were also detected in the coated sample at 1635, 1512, 1470 cm^{-1}. In addition, characteristic signals corresponding to lignin structural units – guaiacyl (G), syringyl (S) and p-hydroxylphenyl (H) were observed in the spectral region from 1028 to 1360 cm^{-1} [15].

The appearance of these characteristic absorption bands in the spectrum of the coated gauze fabric indicates that lignin was successfully dispersed and stably deposited onto the gauze fabric surface via the lignin/DES dispersion system.

4. CONCLUSION

This study successfully demonstrated the feasibility of recovering lignin from coconut coir using a choline chloride – oxalic acid deep eutectic solvent (DES) system. The highest lignin recovery yield (18.70%, w/w) was obtained at a ChCl/OA molar ratio of 6:4, extraction temperature of 100 °C, and extraction time of 3 hours. The obtained lignin showed high purity (73.16 – 86.61%). Furthermore, the recovered lignin exhibited antioxidant activity with an IC_{50} value of 6.02 µg/mL and showed antibacterial activity when dispersed in the DES system against both *Escherichia coli* and *Staphylococcus aureus*. These properties suggest that lignin recovered from coconut coir may serve as a promising bioactive material for value-added applications. However, the recovery efficiency of the DES was not fully quantified, and the evaluation was limited to ensuring that the mass of the recycled DES remained unchanged throughout successive reuse cycles. Overall, the results demonstrate that reusable DES systems provide a sustainable approach for lignin recovery from coconut coir, contributing to the valorization of agricultural residues and supporting the development of green biomass – processing technologies.

REFERENCES

[1] S. W. Adkins, R. Cave, and F. C. Beveridge, "An introduction: Botany, origin and diversity," in *The Coconut: Botany, production and uses*, 2024, pp. 1–13, doi: https://doi.org/10.1079/9781789249736.0001.

[2] A. James and D. Yadav, "Valorization of coconut waste for facile treatment of contaminated water: A comprehensive review (2010-2021)," *Environ. Technol. Innov.*, vol. 24, p. 102075, Nov. 2021, doi: https://doi.org/10.1016/j.eti.2021.102075.

[3] M. Nissar, K. N. Chethan, Y. A. Birjerane, S. Patil, S. Shetty, and A. Das, "Coconut coir fiber composites for sustainable architecture: A comprehensive review of properties, processing, and applications," *J. Compos. Sci.*, vol. 9, no. 10, p. 516, Sep. 2025, doi: https://doi.org/10.3390/jcs9100516.

[4] N. S. M. Aziz, A. Shariff, N. Abdullah, and N. M. Noor, "Characteristics of coconut frond as a potential feedstock for biochar via slow pyrolysis," *Malaysian J. Fundam. Appl. Sci.*, vol. 14, no. 4, pp. 408–413, Dec. 2018, doi: https://doi.org/10.11113/mjfas.v14n4.1014.

[5] D. Rico-García *et al.*, "Lignin-based Hydrogels: Synthesis and applications," *Polymers (Basel).*, vol. 12, no. 1, p. 81, Jan. 2020,doi: https://doi.org/10.3390/polym12010081.

[6] S. Wang, Q. Shen, S. Su, J. Lin, and G. Song, "The temptation from homogeneous linear catechyl lignin," *Trends Chem.*, vol. 4, no. 10, pp. 948–961, Oct. 2022, doi: https://doi.org/10.1016/j.trechm.2022.07.008.

[7] P. Figueiredo, K. Lintinen, J. T. Hirvonen, M. A. Kostiainen, and H. A. Santos, "Properties and chemical modifications of lignin: Towards lignin-based nanomaterials for biomedical applications," *Prog. Mater. Sci.*, vol. 93, pp. 233–269, Apr. 2018, doi: https://doi.org/10.1016/j.pmatsci.2017.12.001.

[8] Y. Jin, J. Lin, Y. Cheng, and C. Lu, "Lignin-based high-performance fibers by textile spinning techniques," *Materials (Basel).*, vol. 14, no. 12, Art. no. 3378, Jun. 2021, doi: https://doi.org/10.3390/ma14123378.

[9] Z. Wang and P. J. Deuss, "The isolation of lignin with native-like structure," *Biotechnol. Adv.*, vol. 68, no. April, Art. no. 108230, Nov. 2023, doi: https://doi.org/10.1016/j.biotechadv.2023.108230.

[10] A. Zeb, "Concept, mechanism, and applications of phenolic antioxidants in foods," *J. Food Biochem.*, vol. 44, no. 9, Art. no. e13394, Sep. 2020, doi: https://doi.org/10.1111/jfbc.13394.

[11] J. L. Espinoza-Acosta, P. I. Torres-Chávez, B. Ramírez-Wong, C. M. López-Saiz, and B. Montaño-Leyva, "Antioxidant, antimicrobial, and antimutagenic properties of technical lignins and their applications," *BioResources*, vol. 11, no. 2, pp. 5452–5481, Mar. 2016, doi: https://doi.org/10.15376/biores.11.2.Espinoza_Acosta.

[12] A. P. Abbott, D. Boothby, G. Capper, D. L. Davies, and R. K. Rasheed, "Deep Eutectic Solvents

Formed between Choline Chloride and Carboxylic Acids: Versatile Alternatives to Ionic Liquids," *J. Am. Chem. Soc.*, vol. 126, no. 29, pp. 9142–9147, Jul. 2004, doi: https://doi.org/10.1021/ja048266j.

[13] D. J. Cronin, X. Chen, L. Moghaddam, and X. Zhang, "Deep eutectic solvent extraction of high-purity lignin from a corn stover hydrolysate," *ChemSusChem*, vol. 13, no. 17, pp. 4678–4690, Sep. 2020, doi: https://doi.org/10.1002/cssc.202001243.

[14] C. Fernandes *et al.*, "New deep eutectic solvent assisted extraction of highly pure lignin from maritime pine sawdust (*Pinus pinaster* Ait.).," *Int. J. Biol. Macromol.*, vol. 177, pp. 294–305, Apr. 2021,doi: https://doi.org/10.1016/j.ijbiomac.2021.02.088.

[15] O. Morozova *et al.*, "Green extraction of reed lignin: The effect of the deep eutectic solvent composition on the UV-shielding and antioxidant properties of lignin," *Int. J. Mol. Sci.*, vol. 25, no. 15, Art. no. 8277, Jul. 2024, doi: https://doi.org/10.3390/ijms25158277.

[16] S. Ferreira *et al.*, "Lignin-based coatings: A sustainable approach to produce antibacterial textiles," *Int. J. Mol. Sci.*, vol. 26, no. 3, Art. no. 1217, Jan. 2025, doi: https://doi.org/10.3390/ijms26031217.

[17] A. D. Sluiter *et al.*, "Determination of structural carbohydrates and lignin in biomass: laboratory analytical procedure (LAP)," *Tech. Rep.*, no. January, pp. 1–15, 2008, [Online]. Available: https://docs.nlr.gov/docs/gen/fy13/42618.pdf.

[18] Suhas, P. J. M. Carrott, and M. M. L. Ribeiro Carrott, "Lignin-from natural adsorbent to activated carbon: A review," *Bioresour. Technol.*, vol. 98, no. 12, pp. 2301–2312, Sep. 2007, doi: https://doi.org/10.1016/j.biortech.2006.08.008.

[19] J. O. Grande-Flores and B. M. Bujanovic, "UV-absorption capacity of selected crude and functionalized lignin for use in sunscreens," *Pertanika J. Sci. Technol.*, vol. 32, no. 3, pp. 1–14, Jul. 2024, doi: https://doi.org/10.47836/pjst.32.S3.01.

[20] A. W. Bauer, W. M. M. Kirby, J. C. Sherris, and M. Turck, "Antibiotic susceptibility testing by a standardized single disk method," *Am. J. Clin. Pathol.*, vol. 45, no. 4_ts, pp. 493–496, Apr. 1966, doi: https://doi.org/10.1093/ajcp/45.4_ts.493.

[21] V. Provost *et al.*, "Deep eutectic solvent pretreatment of biomass: Influence of hydrogen bond donor and temperature on lignin extraction with high β-O-4 content," *Bioresour. Technol.*, vol. 349, Art. no. 126837, Apr. 2022, doi: https://doi.org/10.1016/j.biortech.2022.126837.

[22] N. F. Sayakulu and S. Soloi, "The Effect of Sodium Hydroxide (NaOH) Concentration on Oil Palm Empty Fruit Bunch (OPEFB) Cellulose Yield," *J. Phys. Conf. Ser.*, vol. 2314, no. 1, p. 012017, Aug. 2022, doi: https://doi.org/10.1088/1742-6596/2314/1/012017.

[23] C. Martin *et al.*, "Dilute sulfuric acid pretreatment of agricultural and agro-industrial residues for ethanol production," in *Applied Biochemistry and Biotecnology*, vol. 136, Totowa, NJ: Humana Press, 2007, pp. 339–352, doi: https://doi.org/10.1007/978-1-60327-181-3_30.

[24] D. Mikulski and G. Kłosowski, "Efficiency of dilute sulfuric acid pretreatment of distillery stillage in the production of cellulosic ethanol," *Bioresour. Technol.*, vol. 268, pp. 424–433, Nov. 2018, doi: https://doi.org/10.1016/j.biortech.2018.08.005.

[25] P. Obama, G. Ricochon, L. Muniglia, and N. Brosse, "Combination of enzymatic hydrolysis and ethanol organosolv pretreatments: Effect on lignin structures, delignification yields and cellulose-to-glucose conversion," *Bioresour. Technol.*, vol. 112, pp. 156–163, May 2012, doi: https://doi.org/10.1016/j.biortech.2012.02.080.

[26] T. Yokoyama, "Revisiting the mechanism of β- O -4 bond cleavage during acidolysis of lignin. Part 6: A Review," *J. Wood Chem. Technol.*, vol. 35, no. 1, pp. 27–42, Jan. 2015, doi: https://doi.org/10.1080/02773813.2014.881375.

[27] N. Mqoni, I. Bahadur, S. Singh, X. Meng, and A. Ragauskas, "Deep eutectic solvents for pretreatment of lignocellulose biomass: Physical properties, solubility mechanisms, and their interactions," *Chem. Rev.*, vol. 126, no. 2, pp. 1206–1257, Jan. 2026, doi: https://doi.org/10.1021/acs.chemrev.5c00597.

[28] V. Jančíková and M. Jablonský, "Exploiting deep eutectic solvent-like mixtures for fractionation

biomass, and the mechanism removal of lignin: A review," *Sustainability*, vol. 16, no. 2, Art. no. 504, Jan. 2024, doi: https://doi.org/10.3390/su16020504.

[29] Y. Chen *et al.*, "High-purity lignin isolated from poplar wood meal through dissolving treatment with deep eutectic solvents," *R. Soc. Open Sci.*, vol. 6, no. 1, p. 181757, Jan. 2019, doi: https://doi.org/10.1098/rsos.181757.

[30] M. Francisco, A. van den Bruinhorst, and M. C. Kroon, "New natural and renewable low transition temperature mixtures (LTTMs): Screening as solvents for lignocellulosic biomass processing," *Green Chem.*, vol. 14, pp. 2153–2157, 2012, doi: https://doi.org/10.1039/C2GC35660K.

[31] S. H. Lee *et al.*, "A Review on citric acid as green modifying agent and binder for wood," *Polymers (Basel).*, vol. 12, no. 8, p. 1692, Jul. 2020, doi: https://doi.org/10.3390/polym12081692.

[32] X.-J. Shen *et al.*, "Facile fractionation of lignocelluloses by biomass-derived deep eutectic solvent (DES) pretreatment for cellulose enzymatic hydrolysis and lignin valorization," *Green Chem.*, vol. 21, no. 2, pp. 275–283, 2019, doi: https://doi.org/10.1039/C8GC03064B.

[33] M. Li, M. Tu, D. Cao, P. Bass, and S. Adhikari, "Distinct roles of residual xylan and lignin in limiting enzymatic hydrolysis of organosolv pretreated loblolly pine and sweetgum," *J. Agric. Food Chem.*, vol. 61, no. 3, pp. 646–654, Jan. 2013, doi: https://doi.org/10.1021/jf304517w.

[34] X. Pan *et al.*, "Biorefining of softwoods using ethanol organosolv pulping: preliminary evaluation of process streams for manufacture of fuel-grade ethanol and co-products.," *Biotechnol. Bioeng.*, vol. 90, no. 4, pp. 473–81, May 2005, doi: https://doi.org/10.1002/bit.20453.

[35] V. E. Bécsy-Jakab, A. Savoy, B. K. Saulnier, S. K. Singh, and D. B. Hodge, "Extraction, recovery, and characterization of lignin from industrial corn stover lignin cake," *Bioresour. Technol.*, vol. 399,Art. no. 130610, May 2024, doi; https://doi.org/10.1016/j.biortech.2024.130610.

[36] M. P. Grimaldi *et al.,* "Evaluation of lime and hydrothermal pretreatments for efficient enzymatic hydrolysis of raw sugarcane bagasse," *Biotechnol. Biofuels*, vol. 8, no. 1, Art. no. 205, Dec. 2015, doi: https://doi.org/10.1186/s13068-015-0384-y.

[37] A. Meindl, A. Petutschnigg, and T. Schnabel, "Microwave-Assisted Lignin Extraction—Utilizing Deep Eutectic Solvents to Their Full Potential," *Polymers (Basel).*, vol. 14, no. 20, Art. no. 4319, Oct. 2022, doi: https://doi.org/10.3390/polym14204319.

[38] H. M. Bedair, T. M. Samir, and F. R. Mansour, "Antibacterial and antifungal activities of natural deep eutectic solvents.," *Appl. Microbiol. Biotechnol.*, vol. 108, no. 1, Art. no. 198, Feb. 2024, doi: https://doi.org/10.1007/s00253-024-13044-2.

[39] K. Song *et al.*, "Insights into the chemical structure and antioxidant activity of lignin extracted from bamboo by acidic deep eutectic solvents," *ACS Omega*, vol. 9, no. 39, pp. 40956–40969, Oct. 2024, doi: https://doi.org/10.1021/acsomega.4c06259.

[40] L. Li *et al.*, "A bifunctional brønsted acidic deep eutectic solvent to dissolve and catalyze the depolymerization of alkali lignin," *J. Renew. Mater.*, vol. 9, no. 2, pp. 219–235, 2021, doi: https://doi.org/10.32604/jrm.2021.012099.

[41] Z. Yu *et al.*, "Balancing the particle interactions of lignin-containing cellulose to control dispersibility and viscosity," *Food Hydrocoll.*, vol. 176, Art. no. 112551, Jul. 2026, doi: https://doi.org/10.1016/j.foodhyd.2026.112551.

[42] L. Hu *et al.*, "In situ polymerization of lignin in cellulose nanofibrils aqueous dispersion for fully bio-based barrier coating in packaging," *Cellulose*, vol. 32, no. 9, pp. 5389–5405, Jun. 2025, doi: https://doi.org/10.1007/s10570-025-06562-3.