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Feasibility study of bagasse lignin utilization as an alternative antimicrobial agent

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Abstract

A comparative study was performed using lignin isolated from bagasse dissolved in ethylene glycol (LEG) at 0.5% (w/w) and lignin nanoparticles (LNPs) as an alternative antimicrobial agent. To target safe, commercial use, the LNP preparation conditions involved a simple dialysis of LEG in distilled water for 24 h without chemical modification. This condition yielded 200 nm LNPs with a zeta potential of -39 mV which resulted in good colloidal stability. The original LEG and the obtained LNPs were further tested for their antimicrobial and antioxidant activities. Similar levels of antioxidant activity from both types of lignin compared to gallic acid were obtained based on DPPH radical scavenging testing. The radical scavenging activity levels of LEG and LNPs were 82.4–89.5% and 82.8–91.4%, respectively. Only LEG exhibited positive antimicrobial activity against 5 Gram-positive *Staphylococcus epidermidis* (DMST 15505), *Staphylococcus aureus* (DMST 8840), *Bacillus sp.* (TISTR1323), methicillin-resistant *Staphylococcus aureus* (MRSA; DMDT 20651) and *Staphylococcus aureus* (DMST 8840)) and 4 Gram-negative (*Escherichia coli* (TISTR 117), *Pseudomonas aeruginosa* (TISTR 781), *Pseudomonas fluorescens* (TISTR 358) and *Salmonella typhimurium* (TISTR 1469) bacterial strains, with LNPs being less effective. Regardless of the antimicrobial activity, additional market surveying showed that Thai companies in the preservatives business were interested in an antimicrobial agent in the form of LEG instead of LNPs because the former was easier to handle and had a competitive production cost.

Keywords: Bagasse lignin, Nanoparticle, Antimicrobial activity, Feasibility

1. Introduction

Lignin is currently one of the most attractive biopolymers in both the research and industry communities. Not only is it the second most abundant renewable resource after cellulose, its highly aromatic structure also makes lignin a potential raw material for various applications such as carbon fiber [1,2], polymer composite, UV blocker for cosmetic products [3,4], alternative natural antioxidants and antimicrobial agents to replace synthetic ones [5]. Efficient lignin utilization is still a challenge especially for a high value application due to its heterogeneous chemical structure that may vary depending on the plant source as well as the isolation methods applied. Sugarcane bagasse, a by-product from the sugar industry, is one of the major agricultural by-products in Thailand that could be a source of approximately 7.5 megaton of available lignin annually [6]. Though sugarcane bagasse is currently utilized for energy and electricity production, much attention has also been paid to research and development of value-added products from bagasse lignin as there is an abundant amount of raw material available from Thailand's sugar industry which would be suitable for large-scale production [7]. In addition, it can be extracted using more ecological- and environmental-friendly conditions compared to hardwood and softwood [8].

Technical lignin (kraft, lignosulfonates and organosolv lignin) and lignin extracted from different biomass sources including bagasse lignin have been proved for their antioxidant and antimicrobial activities against both

Gram-positive and Gram-negative bacteria including yeast [9,10]. Various forms of lignin applications as antimicrobial agents have been studied including composite food packaging film with other biomaterial (cellulose, hemicellulose and chitosan) and synthetic materials (polyvinyl alcohol) to prevent pathogenic and food spoilage microorganisms [11,12], lignin coated textile [13] and lignin-loading hydrogels for wound treatment [14]. This makes lignin a good candidate as a natural antimicrobial agent to replace metal nanoparticles, especially silver nanoparticles (SNPs), while its impacts on human health and the environment are inconclusive [15].

Recently, much attention has been paid to lignin nanoparticle (LNP) preparation and the obtained characteristics have demonstrated that on a nanometer scale, lignin physical and chemical properties can be altered to provide a more stable and better-performing material. By definition, a nanoparticle usually refers to a nano-object which has all three external dimensions in the range 1–100 nm [16,17]. The complex and heterogeneous structures of lignin play important roles regarding the sizes, shapes and properties of LNPs obtained. While the research and development to obtain stable LNP properties and scalable methods is still on-going, most of the work dealing with LNP production method development has simply indicated the sizes of the produced material in the ranges of nanometers (< 1 micrometers) or micrometers (1-1000 micrometers), regardless of the various definitions of nanoparticles [18].

Straightforward and environment-friendly methods for LNP preparation have been widely studied. Both simple mechanical and chemical preparation methods have been proposed with highlighted advantages and disadvantages. Generally, mechanical methods involve a reduction in the lignin particle size in water using shear force (ultrasonication or high shear homogenization). Though considered safe and generating small-sized LNPs, this method consumes a substantial amount of energy [19,20]. Chemical preparations based on solvent-lignin-antisolvent reaction with or without dialysis are more favorable as they allow control over the size of the LNPs [21]. In some cases, chemical modification and solubilization in harmful organic solvents like tetrahydrofuran has been used to obtain desired LNP properties and to increase lignin solubility [22].

The current study applied a simple technology to produce an antimicrobial agent from bagasse lignin involving LNPs and explored the properties of the LNPs compared to bulk lignin. The aim was to enhance the use of a natural antimicrobial agent instead of chemical preservatives. Synthetic chemicals have been widely used as antimicrobial agent in Thailand for various products; however, natural compounds are unfamiliar to industry though researchers have spent considerable effort in trying to produce natural antimicrobial agents from different types of biomass. Thus, study investigated determining the perception of Thai industry to using natural antimicrobial agent produced from sugarcane bagasse lignin to better understand the factors influencing the commercialization of a lignin-derived product through a feasibility study.

In this work, the lignin isolated from sugarcane bagasse was prepared in two forms. The first used a solution of lignin dissolved in ethylene glycol (LEG). The second one prepared LNPs by subjecting LEG to dialysis in water with no chemical modification. The obtained LNPs were further characterized for particle size, morphology and stability over time. The antimicrobial activity levels of the LEG and LNPs were determined, with commercial silver nanoparticles (SNPs) used as a reference. The technical and financial feasibility was studied of developing an antimicrobial agent derived from lignin. The production cost on a laboratory scale was calculated and technical information of the obtained product was collected. Recent information on the antimicrobial agent market was gathered from the Thai private sector involved in antimicrobial agent utilization. Then, the interest in using lignin as an alternative natural antimicrobial agent by Thai industry was analyzed.

2. Materials and methods

2.1 Bagasse lignin extraction

The sugarcane bagasse was kindly provided by Mitr Phol Sugar Factory (Chaiyaphum province, Thailand) from the 2018 harvesting season. Before lignin extraction, the bagasse was dried in a ventilated oven at 60 °C for 24 h. The lignin was isolated from the dried bagasse under mild conditions of alkali hydrolysis with dried bagasse to a 1%NaOH solution ratio of 1:10 (w/v). The mixture was heated to 110 °C for 1 h using an autoclave. After bagasse removal using filtration, the pH of the filtrate was adjusted to 2 with 50% H_2SO_4 and the precipitated lignin was separated through filter paper. The precipitated lignin was washed with deionized water 4 times until the pH of the filtrate was stable (around 3–3.5). The filtered lignin was dried in a ventilated oven at 50 °C overnight for further use.

2.2 Preparation of lignin solution in ethylene glycol (LEG)

Lignin powder was solubilized in ethylene glycol (EG) at various concentrations (0.5, 1, 2.5 or 5% wt). The solution was stirred using a magnetic stirrer for 2 h at room temperature. Each solution was centrifuged at 10,000 rpm and 20 °C for 10 min to remove any insoluble parts.

2.3 Preparation of lignin nanoparticles (LNPs)

The dialysis process was used to produce LNPs. A volume of 100 mL of each LEG solution was transferred to a dialysis bag (Spectra Por® 1 Standard RC Tubing, 6-8 kDa MWCO, Spectrum Labs, USA) and immersed in excess volume of distilled water under low speed stirring. The water was periodically changed during 24 h. The particle sizes of the LNPs prepared from the different pre-dialysis lignin concentrations were compared.

2.4 Particle size and zeta potential measurement

The average particle size and zeta potential of the LNPs were measured using a Nanoparticle analyzer (NanoPlus HD, Particulate systems, USA).

2.5 Scanning electron microscopy (SEM)

A drop of colloidal LNPs was placed on a sample holder and dried at room temperature. The dried sample was coated with a layer of gold. The morphology of the LNPs was observed using SEM (Quanta 450FEI) at 20 KV.

2.6 Antimicrobial acitivity test

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of lignin samples were determined for both Gram-positive and Gram-negative bacteria. The bacterial strains were obtained from the Department of Medical Sciences Thailand (DMST) and the Thailand Institute of Scientific and Technological Research (TISTR). The tested Gram-positive bacterial strains were: *Staphylococcus epidermidis* (DMST 15505), *Staphylococcus aureus* (DMST 8840), *Bacillus sp.* (TISTR1323), methicillin-resistant *Staphylococcus aureus* (MRSA; DMDT 20651) and *Staphylococcus aureus* (DMST 8840). The Gram-negative bacterial strains were: *Escherichia coli* (TISTR 117), *Pseudomonas aeruginosa* (TISTR 781), *Pseudomonas fluorescens* (TISTR 358), *Salmonella typhimurium* (TISTR 1469) and *Propionibacterium acnes* (DMST 14916). The method described by Basri and Fan [23] was adopted. In brief, all bacterial strains were cultured on tryptone soy agar (TSA) and incubated for 24 h at 37 ºC. The bacterial suspension from all strains was prepared in sterile 0.85% NaCl solution and the turbidity was adjusted to 0.5 McFarland's standard (approximate cell density at 1×108 CFU/mL). The bacterial suspension was further diluted in Muller-Hinton Broth (MHB) using the ratio of 1:200 (v/v). A hundred microliters of each tested sample (0.1% SNP, 0.5% LNPs and 0.5% LEG) was twofold serially diluted with MHB from the initial concentration and mixed with 100 µl of bacterial suspension (1×10^5 CFU) in 96-well microplates and incubated for 24 h at 37 ºC. The control was a mixture of broth and bacterial suspension. The concentration of each sample that changed the color of resazurin solution after being added into the bacterial suspension was identified as the MIC. The MBC was determined by subculturing the well showing no apparent growth in a sterile TSA agar plate and the concentration that had no bacterial growth on the plate was defined as the MBC.

2.7 Antioxidant activity test

DPPH radical scavenging activity (RSA) of LEG and LNPs was tested based on the method proposed by Fagali and Catala [24]. The LEG and LNP samples at a concentration of 1 $mg.mL^{-1}$ were prepared in EG and water, respectively. A volume of 1 mL of sample was mixed with 1 mL of 0.25 mM DPPH dissolved in ethanol. The absorbance was measured at 517 nm against a blank of sample and ethanol mixture (1:1, v/v) for 30 min to observe the kinetics. A mixture of 0.25 mM DPPH and ethanol at 1:1 (v/v) was used as the control. Gallic acid at the same concentration of sample was used for comparison. The measurement was done in 3 replicates. The radical scavenging activity was calculated using Equation (1):

$$\%RSA = (A_{control} - A_{sample})/ A_{control}) \times 100 \quad (1)$$

where $A_{control}$ and A_{sample} are the absorbance levels of the control and sample, respectively.

2.8 Technical and financial feasibility study and market survey

The technical information of the production method, characteristics and properties of the LEG and LNPs were collated. The financial feasibility was determined based on the production cost at the laboratory scale. The calculation included direct materials, direct labor and manufacturing overheads during processing. The technical information of the LEG and LNPs including production cost was included in a questionnaire used in the market

survey. The Thai industrial sector participated in this study, involving antimicrobial agent traders and cosmetic producers from 35 companies. The marketability of bagasse lignin utilization as an alternative antimicrobial agent was determined from the surveyed information.

3. Results and discussions

3.1 Characteristics of LNPs from different LEG concentration

Lignin extracted from sugarcane bagasse was dissolved in EG (LEG) at different concentrations in the range 0.5–5% wt. The LEG solution was further dialyzed in water for 24 h. The lignin nanoparticles (LNPs) were obtained by decreasing the lignin solubility in EG in the presence of water [18]. The average size of the LNPs from each LEG concentration was determined using a nanoparticle analyzer, and the morphology was investigated using SEM. The results showed that the average size of the LNPs substantially increased from 200 to 800 nm with an increase in the pre-dialysis LEG concentration, as shown in Figure 1 (A). Similar results were observed with the LNPs prepared using different lignin types or different solvents or both [25].

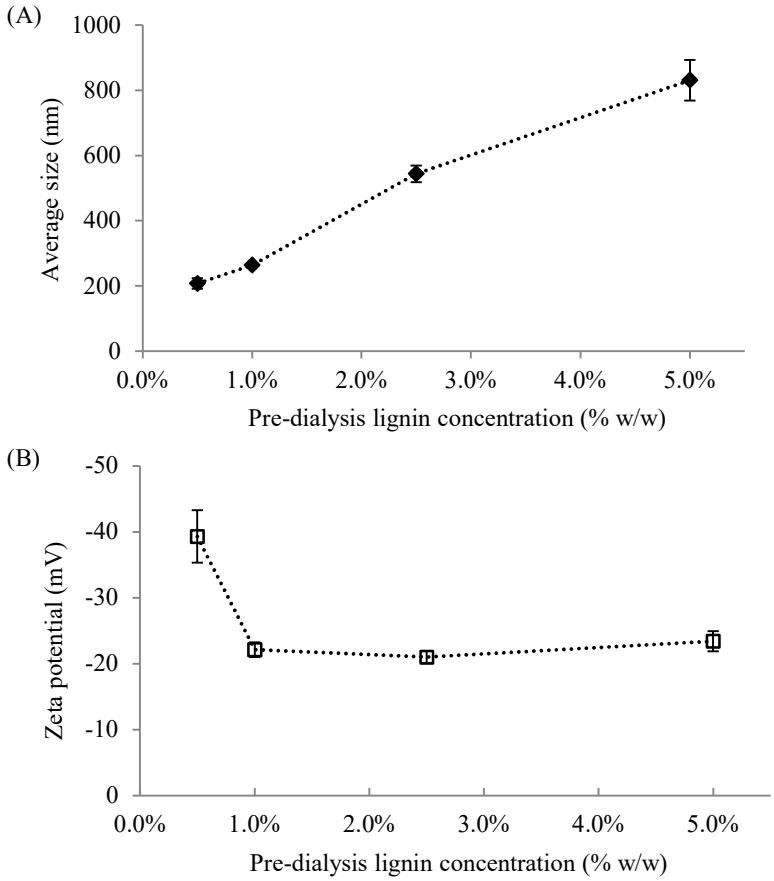

Figure 1 Average sizes (A) and zeta potential (B) of LNPs prepared from different pre-dialysis lignin concentrations of 0.5, 1, 2.5 and 5%wt and the error bars indicated ± standard deviation.

The absolute value of the zeta potential (ζ) decreased with increasing the initial LEG concentration. At 0.5% concentration, ζ was - 39 mV while for the 5% LEG concentration, it was -22 mV (Figure 1 (B)). The morphology of the LNPs from the different initial LEG concentrations was investigated using SEM. This preparation process yielded spherical nanoparticles, as observed from the images in Figure 2. Agglomeration of LNPs was noticed at the higher initial LEG concentration (Figures 2c and 2d) which would explain the decreased zeta potential of the LNPs from the higher lignin concentration, since particle agglomeration could reduce the repulsive forces. The colloidal stability over storage for 9 month under ambient conditions was observed for the LNPs obtained from 0.5% LEG (data not shown).

Solvent was one of the factors reported to influence particle shape. Beisl et al. [21] reported that most technical lignin (kraft lignin (KL), softwood KL and organosolv) dissolved in EG prior to dialysis, resulting in

irregular-shaped nanoparticles. Dissolution of lignin in tetrahydrofuran (THF), acetone/water mixture and dimethylsulfoxide resulted in solid, spherical particles. However, the spherical shape of the bagasse lignin in EG solution observed using SEM was not in agreement with previous work [18].

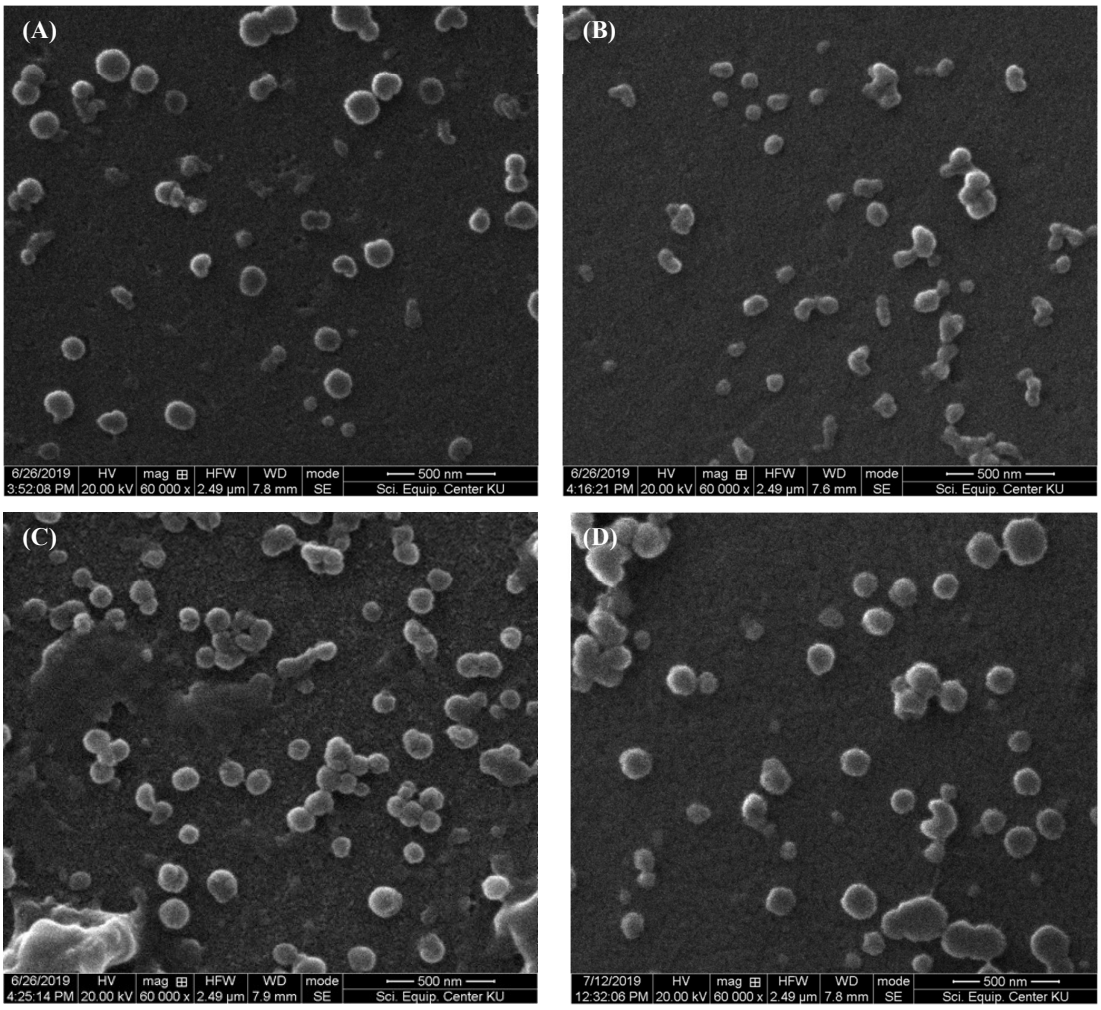

Figure 2 SEM images of LNPs from pre-dialysis lignin concentrations of 0.5% (A), 1% (B), 2.5% (C) and 5% wt. (D).

3.3. Antimicrobial activity of LEG and LNPs

The LEG at 0.5% concentration, LNPs obtained from dialysis and the commercial SNP (1%) were tested for antimicrobial activity based on their MIC and MBC values. SNP was the most effective antimicrobial agent with minimum values of MIC and MBC in the ranges 19.53–625 µg.mL^{-1} and 39.06–625 µg.mL^{-1}, respectively. A positive result was observed for 0.5% LEG with MIC values in the range 625–1,250 µg.mL^{-1} and an MBC value of 1,250 µg/mL^{-1}. Compared to the SNPs, LEG was effective against almost every bacterial strain tested except *P. acnes*. This strain seemed more resistant than the other tested strains as indicated by its higher MIC value. The antimicrobial activity of EG was also determined. EG alone has been reported to inhibit the growth of *E. coli* at 24% (v/v culture broth) concentration within 4 h [26]. In the current study, a slight antibacterial effect of pure EG was observed with MIC and MBC values of 125,000 and 250,000 µg.mL^{-1}, respectively. Nevertheless, in the lignin solution (LEG 0.5%) with the presence of EG at 99.5% (w/w), the major antimicrobial effect of LEG was mainly due to the lignin as the MIC value was much lower than for EG. For the LNPs, no antimicrobial activity was detected for any bacterial strain, even though it was prepared from LEG that positively inhibited microbial growth. This inability of the LNPs to exhibit antimicrobial activity could have been linked to their characteristics including surface charge, size and shape of the particles.

Other studies dealing with the antimicrobial activity of metal nanoparticle also described the crucial effect of the surface charge. For example, Abbaszadegan et al. [27] reported that positive, neutral and negative surface

charges on SNPs from different preparation methods resulted in different levels of antimicrobial activity. The positively charged SNPs had the highest antimicrobial activity against both Gram-positive and Gram-negative bacteria, while the negatively-charged SNPs had the least activity. Similar results were observed with iron oxide nanoparticles (IONPs). Modification of the negative surface IONPs using a chitosan coating method reversed the surface charge to positive and resulted in higher antimicrobial activity [28]. The better inhibitory effect from positively charge particles could have been due to the fact that the net charge of bacterial cells is negative, so better attachment between the antimicrobial agent and cells could be obtained.

Nevertheless, the surface charge was not the only parameter influencing this activity. Yang et al [29] showed that LNPs obtained from the dialysis of pristine lignin dissolved in EG and when chemically modified using different acid types could inhibit the growth of plant pathogenic bacteria even though the zeta potential was in the range -20 to -30 mV. This could be plausibly explained by the fact that the smaller particle size of the LNPs in the current study (50-120 nm) could better disrupt the bacterial membranes; consequently, this led to membrane damage. The ability of nanoparticle-based antimicrobials is commensurate with size. The smaller particles with a greater surface-volume ratio could increase the interaction with the bacterial cell wall and membranes. The efficiency could be also tuned based on the size; Hayden et al [30] reported that reduction of the particle size of cationic gold particles from 6 to 2 nm enhanced bacterial cell lysis ability. Furthermore, the shape of the particle affected antimicrobial activity as observed from the better inhibition of bacterial biofilm formation from the rod-like-shaped silica nanoparticles compared to spherical ones [31]. It can be concluded that the contact between NPs and bacterial cells is important to achieve antibacterial activity and the LNP characteristics obtained under the current study conditions can be used to tune the LNPs for use as an antimicrobial. Nevertheless, several reports indicated the positive antimicrobial ability of LNPs when infused with metal ions such as silver, zinc and titanium as reducing, capping and stabilizing agents [32-34]. Considered that the environmental impact of metal ions used to prepare the nanoparticles (especially the silver ion) was still inconclusive, with this approach, the load of metal ions can be reduced.

Table 1 MIC and MBC values of commercial SNP (0.1% wt), LNPs (0.5%wt), lignin in EG (0.5%wt) and ethylene glycol (EG) against Gram-positive and Gram-negative bacteria.

Bacterial strain	MIC ($\mu g.mL^{-1}$)				MBC ($\mu g.mL^{-1}$)			
	SNP	LNP	LEG	EG	SN	LNP	LEG	EG
E. coli (-)	39.06	ND	1,250	250,000	78.12	ND	ND	250,000
P. aeruginosa (-)	19.53	ND	625	125,000	78.12	ND	1,250	250,000
P. fluorescens (-)	39.06	ND	625	125,000	625	ND	ND	125,000
S. Typhimurium (-)	39.06	ND	625	125,000	78.12	ND	1,250	125,000
P. acnes (-)	625	ND	ND	ND	625	ND	ND	ND
S. epidermidis (+)	78.12	ND	1,250	250,000	312.2	ND	ND	250,000
Bacillus sp. (+)	39.06	ND	1,250	250,000	312.5	ND	1,250	250,000
M. luteus(+)	39.06	ND	625	125,000	78.12	ND	1,250	250,000
S. aureus (MRSA) *(+)*	19.53	ND	625	125,000	39.06	ND	ND	125,000
S. aureus (+)	19.53	ND	625	125,000	78.12	ND	1,250	250,000

(+ / -) indicates Gram-positive / Gram-negative bacteria.

ND: not detected.

3.4 Antioxidant activity

The antioxidant activity was verified using DPPH assay to determine the effect of nanoparticle preparation. The test was performed using the initial LEG solution. Colloidal LNPs were centrifuged to remove the water and then dried at 50 °C. The obtained LNP powder was resuspended in water (LNP-W) using homogenizer at 5,000 rpm for 6 min and another sample was made by dissolving in EG (LNP-EG) with stirring for 2 h using a magnetic stirrer. The concentrations of all samples and of the gallic acid standard were adjusted to 1 $mg.mL^{-1}$. The radical scavenging activity (%RSA) of every sample is shown in Table 2. It was observed that the gallic acid scavenged most of the DPPH radical (93.2%) within the first minute. Similarly, the %RSA of LNP-W (~ 90%) remained constant from 1 to 30 min of measurement. Both the lignin in EG either prepared from the original lignin and from the LNP powder reacted to the DPPH radical more slowly. A significant difference was observed in the %RSA values at the beginning and after 30 min of reaction. These results suggested that in the colloidal state (LNP-W), the antioxidant activity of nanoparticles could be maintained compared to the parent material in EG solution. Furthermore, the instant reactivity was close to that for pure antioxidant molecules which was in accordance with the advantageous use of lignin at the nanometer scale, as described previously.

Table 2 Radical scavenging activity (%RSA) of LEG, LNP-EG, LNP-W and gallic acid standard at 1 $mg.mL^{-1}$ during 30 min of measurement.

%RSA	LEG	LNP-EG	LNP-W	Gallic acid
at 1 min	82.4 ± 1.19^{c}	82.8 ± 1.67^{c}	87.5 ± 1.39^{a}	93.2 ± 0.17^{a}
at 5 min	87.0 ± 0.58^{b}	88.2 ± 0.93^{b}	88.8 ± 1.56^{a}	93.2 ± 0.14^{a}
at 10 min	88.3 ± 0.47^{ab}	89.7 ± 0.65^{ab}	89.5 ± 1.47^{a}	93.2 ± 0.12^{a}
at 20 min	89.2 ± 0.41^{a}	90.8 ± 0.54^{a}	90.4 ± 1.53^{a}	93.2 ± 0.12^{a}
at 30 min	89.5 ± 0.36^{a}	91.4 ± 0.57^{a}	90.7 ± 1.56^{a}	93.3 ± 0.04^{a}

Mean of 3 repetition ± standard deviation.

3.5 Financial and market feasibility study of using lignin as antimicorabial agent

The information of the production cost including lignin extraction, dissolution in EG and LNP preparation using dialysis in a laboratory was collected to determine financial feasibility. The cost structure of LEG production is presented in Table 3. The total cost of LEG production was USD 26.98/L, and up to 93% of the cost was materials used and the main expense was on EG (47.5%). If EG is considered as an antimicrobial agent due to its slight activity, adding 0.5% lignin in EG could reduce the effective concentration by 200 times, as shown in Table 1. For this reason, the cost of the bagasse lignin solution was lower than that of EG in terms of efficiency.

Table 3 Cost structure per liter LEG 0.5% (w/w) for laboratory-scale production.

List		Cost (USD)	Proportion (%)
Raw material/Quantity used		25.3	93.8%
Dry bagasse lignin	5.6 g	7.02	26.0%
Ethylene glycol	1 L	12.81	47.5%
Filter paper	11 pcs	5.47	20.3%
Labor		1.2	4.4%
Utility		0.47	1.7%
Depreciation		0.02	0.1%
Total		26.98	100%

Remarks: Foreign exchange rate at 28 August 2020: THB 31.226 = USD 1.

LEG solution was further used to prepare LNPs and the details of cost are presented in Table 4. Though the property of LNPs as antimicrobial agent must be improved the calculation of production cost was useful. It could be referred to as the minimum cost of antimicrobial in LNP form. The total cost of LNP production was USD 153.34 L^{-1} and the major cost was for dialysis bags (up to 63% of the total cost). The high cost of the dialysis step was a limitation for LNP scaling up though its advantage of having better control over the particle dispersion concentration, compared to the drop-by-drop method, was conclusive [25]. Mechanical methods such as dry and wet milling were simply used to reduce particle size, but non-uniformity in the shape and broad size distributions were the main disadvantages especially when specially-tailored structures were targeted [18].

Table 4 Cost structure per liter LNPs for laboratory-scale production.

List		Cost (USD)	Proportion (%)
Raw material/Quantity used		147.88	96.4%
Bagasse lignin solution	1 L	26.98	17.6%
Distilled water	150 L	24.02	15.7%
Dialysis bag	2.5 m	96.87	63.2%
Labor		1.2	0.8%
Utility		4.03	2.6%
Depreciation		0.23	0.1%
Total		153.34	100.0%

Remarks: Foreign exchange rate 28 August 2020: THB 31.226 = USD 1.

General information on the utilization of antimicrobial agent or preservative for industrial use was obtained from the questionnaires distributed to 35 companies in Thailand to obtain current market information. The main

type of company participating in the study were traders (88.6%) and the remainder were cosmetic producers. The information on the preservative market indicated that 75% of preservatives in Thailand were produced domestically while 25% were imported. Among the imported products, one-half (51%) was from China. Up to 90% of the preservatives in Thailand were used for cosmetic products. The two commercial synthetic preservatives widely used in cosmetic products were phenoxyethanol and chlorphenesin with proportions of 75% and 25%, respectively. These chemicals were used based on their effectiveness as antimicrobial agents at low dose and phenoxyethanol was more popular due to its lower price (USD 41.6 kg^{-1}) compared to chlorphenesin (USD 176 kg^{-1}).

The responses provided in the questionnaires indicated there was interest in new natural preservatives or antimicrobial agents targeted for industrial use. With the technical information on the two forms of lignin (pH, lignin concentration, solubility, activity, stability and price) given to the respondents, 75% of them were concerned about safety and they wanted more background research of the product before they could consider it further. Regardless of the backup information, the price and the form of product were the first and the second important criteria, respectively. The acceptable price of the new product should be in the range USD 25.62–32.02 kg^{-1}. The preferred form of antimicrobial agent was as a liquid or solution for easy handling as further preparation was usually required for solid or powder forms. Therefore, LEG was considered by the private sector to be a potential candidate as an alternative antimicrobial agent, with 80% of the companies expressing interest, mostly due to the low production cost. For LNPs, further modification to improve its antimicrobial activity and cost efficiency for it to be competitive with chemical preservatives. In terms of production, the process to scale up LEG production to a commercial scale was considered feasible because it was simple, involving just the dissolution of lignin in a solvent. However, there remained a technical challenge for lignin solubility as it was mostly solubilized in organic solvent and the solubility was also limited which may hinder the application. In addition, EG is rarely used; therefore, increasing lignin solubility in a solvent that is recognized as safe for end-use products should be explored.

4. Conclusion

Using the simple preparation method of solubilizing lignin in EG, successful antimicrobial activity was achieved against 10 strains of both Gram-positive and Gram-negative bacteria. Preparation in the LNP form based on dialysis in distilled water negatively affected the antimicrobial activity as the LNPs could not inhibit antimicrobial growth while their antioxidant activity and colloidal stability over 9 month of storage was preserved. The performance of nanoparticle antimicrobials has been reported to be strongly related to the particle size as well as the surface charge. It could be concluded that surface modification is necessary to enhance the antimicrobial activity of LNPs. To gauge producer perceptions of using antimicrobial agents made from lignin, 35 Thai companies involved in antimicrobial agent utilization were surveyed. The results suggested that from an industrial point of view, a competitive price was critical. In addition, background research of the product was required to ensure it would be safe to use. Notably, besides the technological investment that may be required for advance use of lignin, technology must be simple for commercialization, especially to limit the cost of production. LEG was a possible substitute for synthetic antimicrobial agents but further efficiency studies are required with precise application.

5. Acknowledgments

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6. Conflict of Interests

The authors declare no conflict of interest.

7. References

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