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Antioxidant and antibacterial activities of *Pandanus amaryllifolius* Roxb. (Pandanaceae) prop roots and its application for a novel bacterial cellulose (*Nata*) fermentation by enzymatic hydrolysis

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ABSTRACT

Pandanus amaryllifolius Roxb. (Pandanaceae) prop root was investigated for biological activities, i.e. antioxidant (DPPH radical scavenging assay) and antibacterial activity against *Staphylococcus aureus* DMST 2933 and *Escherichia coli* DMST 4212. The results showed that a crude extract of pandan prop roots exhibited antioxidant activity with IC_{50} of 230.24 ± 10.69 µg/ml, and it had a total phenolic content of 24.75 ± 0.74 mg GAE/g of TPC content and inhibited the growth of *S. aureus* DMST 2933 with 9.75 ± 0.35 mm of inhibition zone diameter. The prop root powder was used to develop a novel bacterial cellulose (BC) production using enzymatic hydrolysis. The maximum total soluble solids content at 2.67 ± 0.29 Brix was found when using prop root powder at 100 g/l with 4.0% (v/v) of the commercial enzyme (iKnowZyme® cellulase) after incubated at 50°C, pH 5.0 for 24 h. The hydrolysis pandan prop root was fermented at room temperature for nine days with *Komagataeibacter xylinus* AGR 60, yielded 13.5 ± 0.50 mm of thickness with 7.90 ± 0.10 g of dry weight. Scanning electron microscope and Fourier-transform infrared spectroscopy were used to characterize the physical and chemical structure of the BC produced from pandan prop root, revealing that pandan prop root has the potential for a novel BC production with bioactivities of antioxidant and antibacterial properties.

1. INTRODUCTION

Pandanus amaryllifolius Roxb. is a tropical plant in the Pandanaceae family which usually used as a source of natural coloring, flavoring, and bioactive compounds in food additives and the pharmaceutical industry [1,2]. Clemen-Pascual *et al.* [3] reported that pandan leaves contain alkaloids, flavonoids, glycosides, and tannins that can be used to reduce fever, antibacterial and anticancer. Moreover, the pandan leaves also contained polyphenols with antioxidant and antihyperglycemic activities [4,5]. However, information about the bioactivities and applications of pandan prop root is scarce since it is discarded as waste. Then it is interesting to study and utilize for value-added through the biotechnological process. Bhuyan and Sonowal [6] reported that pandan prop root contained various bioactive compounds such as phenolic compounds and flavonoids, which were able to scavenge the free superoxide radicals as anti-aging and anticancer properties. Peungvicha *et al.* [7] found that pandan prop root extracts prolonged the sleeping time and reduced the locomotor activity in mice, while Jimtaisong and Krisdaphong [8] reported on the antioxidant properties of pandan root and bioactivities for the possible development of novel healthy foods or functional products in the future.

Bacterial cellulose (BC) is composed of β-1,4 linkages of glucose polymer $(C_6H_{10}O_5)n$ and can be produced from various bacterial species, including *Komagataeibacter* [9]. BC is applied in foods, medicines, and cosmetic products [10], which were produced from various substrates such as coconut juice, fruit juice, rice syrup, and industrial waste [11,12]. A novel substrate for BC production will provide an alternative BC product that generates the unique functions and bioactivities which has not been found in the conventional substrate [13]. The pandan leave syrup was currently supplemented with the BC produced from low-grade rice as the flavoring [14]. But from our knowledge, the BC production from pandan prop root has not yet been reported, which is interesting to provide a novel BC from the pandan prop root in this study.

Here, the bioactivities of pandan prop root include antioxidant DPPH radical scavenging assay, total phenolic content (TPC), and antibacterial activities against *Staphylococcus aureus* DMST 2933 and *Escherichia coli* DMST 4212 were investigated, together with the development of a novel BC product from pandan prop root using enzymatic hydrolysis.

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2. MATERIALS AND METHODS

2.1. Substrates, Enzyme and Bacterial Strain

Organic pandan prop root was obtained from Chachoengsao Province, Thailand. The prop root was washed twice in tap water, dried at 50°C in a hot air oven for 12 h, then powdered by an electric grinder and stored under dry condition. Commercial cellulase enzyme (iKnowZyme® cellulase) was purchased from Reach Biotechnology Co., Ltd. and stored at -20°C. *Komagataeibacter xylinus* AGR 60 was obtained from the Institute of Food Research and Product Development, Kasetsart University, Thailand. The AGR 60 strain was grown in a coconut juice inoculum medium reported by Noree *et al.* [13] and used as the starter culture for BC fermentation.

Pathogenic bacteria, including *S. aureus* DMST 2933 and *E. coli* DMST 4212, were obtained from the Department of Medical Sciences Culture Collection Center, Ministry of Public Health, Thailand, and grown on TSB medium at 37°C for 24 h and diluted to 10^6 CFU/mL for disc diffusion assay.

2.2. Chemical Composition Analysis of Pandan Prop Root

Proximate analysis of pandan prop root including protein, fat, moisture, and ash contents was analyzed by the Central Laboratory (Thailand) Co., Ltd. following the method of analysis for nutrition labeling (1993) p.106 and AOAC methods [15]. Hemicellulose, cellulose, and lignin were analyzed using AOAC methods [15]. All values were reported as g/100 g sample.

2.3. Methanol Extraction of Pandan Prop Root

Pandan prop root powder at 500 g was immersed in 1.0 L of 95% methanol at room temperature for 24 h. The extract solution was filtered using Whatman No.1 filter paper, and the solvent was removed using a rotary evaporator at boiling point [16]. The obtained pandan prop root was used to determine antioxidant (DPPH radical scavenging assay) and antibacterial activity on *S. aureus* DMST 2933 and *E. coli* DMST 4212 by the disc diffusion method.

2.4. Determination of Antioxidant Activity

2.4.1 DPPH radical scavenging assay

The antioxidant assay was reported as the amount of antioxidant with the ability to reduce DPPH absorption compared to the control (IC_{50}), defined in units of µg/ml, following the modified procedure of Suwannakul *et al.* [17]. A reduction in DPPH concentration was recorded by the decrease in absorbance at 520 nm. Ascorbic acid was used as the positive control with ethanol as the negative control and extract without DPPH as the blank.

2.4.2 TPC

The TPC of pandan prop root extract was determined by the Folin-Ciocalteu method as described by Butsat and Siriamornpun [18]. Diluted Folin-Ciocalteu reagent (1:10) was added to 200 µL of the sample. Then, 800 µL of sodium carbonate (Na_2CO_3) was added, and the reaction volume was increased to 5.0 mL by distilled water. The reaction was incubated at room temperature for 2.0 h and measured at 760 nm. Gallic acid was used as the standard reagent, with results expressed as mg gallic acid equivalent per g of sample (mg GAE/g sample).

2.5. Antibacterial activity

To determine the *in vitro* activity of prop root crude extract against pathogenic bacteria, including *S. aureus* DMST 2933 and *E. coli* DMST 4212, prop root crude extracts at 12.5, 25, 50, and 100 mg/ml were incorporated in a 6.0 mm paper disc and tested using the paper disc diffusion method [19]. The testing plates were incubated at 35 ± 2°C for 18 h to determine the mean inhibition zone of each sample (mm). The extracted solvent was used as a control.

2.6. Hydrolysis of Pandan Prop Root by Commercial Hydrolytic Enzyme

Pandan prop root hydrolysis reactions were investigated in 250 mL Erlenmeyer flasks containing 50 mL of enzyme solution. Pandan prop root powder content was varied at 25–150 g/l in the reaction containing iKnowZyme® cellulase concentration at 4.0% (%v/v) dissolved in 0.1 M acetate buffer pH 5.5. The reactions were incubated at 50°C for 24 h, modified from Kobkam *et al*. [20] and determined for total soluble solids (TSS) content using a refractometer (RA-250WE, Kyoto Electronics, Kyoto, Japan). The optimum substrate concentration was used, with enzyme concentration varied at 1.0–6.0 (%v/v). The reactions were incubated at 50°C for 24 h and determined for TSS content as described above. The optimum substrate and enzyme concentrations were used to upscale the hydrolysis in a 5.0 L glass jar chamber for sugar syrup production.

2.7. Production of BC by *K. xylinus* AGR 60

Sugar syrup obtained from the hydrolysis of pandan prop root was used as the substrate for BC fermentation by *K. xylinus* AGR 60 in a plastic tray (15 × 15 × 6 cm) with the working volume at 500 mL. The fermentation medium was supplemented with 3.0 g of ammonium sulfate and 0.6 mL of glacial acetic acid following the methods of Jagannath *et al*. [21] and Noree *et al.* [13] with slight modifications and 10% (v/v) of *K. xylinus* AGR 60 was used as a starter culture. The fermentation was incubated at room temperature, and the characteristics of BC were determined after nine days of cultivation. To compare with the traditional BC production, coconut juice was used as a substrate. The thickness of each sample was recorded every day. At the end of fermentation, all the BC samples were dried at 50°C for 24 h to determine the dry weight. The structure of freeze-dried BC was determined by a scanning electron microscope (SEM) (model SU8020; Hitachi, Tokyo, Japan) at 10.0 kV, while functional groups and chemical bonds of BC produced from pandan prop root syrup were determined by Fourier-transform infrared spectroscopy (FTIR, Thermo Scientific Nicolet is5, USA) in the range 4000–400 cm^{-1} following the methods of Saleh *et al*. [21] and Noree *et al*. [13].

2.8. Statistical Analysis

The significance of the data was analyzed by one-way analysis of variance (SPSS 21.0, USA). Values were considered significant at $P < 0.05$ using Duncan's multiple range tests.

3. RESULTS AND DISCUSSION

3.1. Chemical Composition of Pandan Prop Root

P. amaryllifolius Roxb. is a monocot that grows in tropical regions, commonly known as pandan. The prop roots of pandan are adventitious and used for aerial support, as shown in Figure 1. The leaves and stems have a unique and pleasant aroma. They are used as flavorings for various foods and herbal beverages and reduce fever, relieve indigestion and as a diuretic, cardio-tonic, and anti-diabetic [22-25]. Bhuyan and Sonowal [6] reported that pandan leaves and roots contained various bioactive compounds such as phenolic compounds and flavonoids, which showed the bioactivities of antioxidant, anticancer, and anti-

aging bioactivities. The pandan prop root was traditionally used for diabetic patients treated with the antihyperglycemic property of 4-hydroxybenzoic acid [8,25]. However, the pandan prop root is usually discarded as waste, which is interesting to utilize as a novel substrate for food and beverage production.

The chemical composition of pandan prop root powder is shown in Table 1. The components were cellulose at 38.88 ± 0.11 g/100g and hemicellulose at 15.40 ± 0.16 g/100g, with small amounts of lignin (8.99 ± 0.03 g/100g), protein (4.67 ± 0.01 g/100g), carbohydrate (80.24 ± 0.11 g/100g), fat (1.11 ± 0.01 g/100g), moisture (7.56 ± 0.03 g/100g) and ash (6.31 ± 0.01 g/100g). Prop roots develop from the nodes to provide additional support for plants, and they contain cellulose as the major component. The high amount of cellulose showed the potential of the substrate for cellulase enzyme hydrolysis to produce fermentable sugar and bioactive compounds. The pandan prop root was investigated for bioactivities and used as a substrate for BC fermentation.

3.2. Antioxidant Activity of Pandan Prop Root Extract

Antioxidant assay of pandan prop root was expressed as the half-maximal inhibitory concentration (IC_{50}) as described in the method. The IC_{50} value of DPPH scavenging activity from pandan prop root crude extract was 230.24 ± 10.69 µg/ml. TPC was 24.75 ± 0.74 mg GAE/g. Suwannakul *et al*. [17] reported the IC_{50} and TPC of pandan leaf extract at 110.57 ± 36.42 µg/ml and 57.25 ± 0.02 mg GAE/g, respectively. Results revealed that pandan prop root contained bioactive compounds and showed antioxidative activities for possible use as an ingredient in functional foods and cosmetic products in the future. At the same time, Jimtaisong and Krisdaphong [8] confirmed that pandan root extract exhibited antioxidant activity of DPPH as IC_{50} value at 2,340 µg/ml and TPC content at 28.8 mg GAE/g that could be used in topical pharmaceutical and cosmetic applications.

Figure 1: *Pandanus amaryllifolius* Roxb. (Pandanaceae) showing leaves and prop roots.

Table 1: Chemical composition of pandan prop root powder.

Component (%)	(g/100g)
Cellulose	38.88±0.11
Hemicellulose	15.40±0.16
Lignin	8.99±0.03
Protein	4.67±0.01
Fat	1.11±0.01
Moisture	7.56±0.03
Ash	6.31±0.01

Values are averages of three determinations.

3.3. Antibacterial Activity

The crude extract of pandan prop root was evaluated for the antibacterial ability of *S. aureus* DMST 2933 and *E. coli* DMST 4212 using the disc diffusion assay method as described above. Results of crude extract at different concentrations on antibacterial activity are shown in Table 2. Crude extract of pandan prop root inhibited the growth of *S. aureus* DMST 2933 in all concentrations (12.5–100 mg/ml), with the highest mean zone of inhibition at 9.75 ± 0.35 mm using 100 mg/ml of crude extract. However, the pandan prop root crude extract showed no in vitro activity against *E. coli* DMST 4212, concurring with Dumaoal *et al*. [19], who reported that pandan leaf crude extract had activity against the growth of *S. aureus* ATCC 25923 but not against the growth of *E. coli* ATCC 25922. Pandan leaf and root contain phytochemicals as bioactive compounds and essential oil that inhibit the growth of some pathogenic bacteria [8,19]. Mar *et al*. [26] found 54 compounds in essential oil from pandan leaves that inhibited the growth of various Gram-positive and Gram-negative bacteria, including *S. aureus*, a common cause of minor skin infections, mainly when introduced into a wound or skin incision. From our knowledge, most of the previous findings focused on the antibacterial activity of pandan leaves. However, the study concurring with pandan prop root is scarce. Then, this study is the first report for evaluating the antibacterial activity of pandan prop root crude extract, which showed the potential for application as an ingredient for skin cosmetic and pharmaceutical products.

3.4. Hydrolysis of the Pandan Prop Root by Commercial Hydrolytic Enzyme

For sugar syrup production, pandan prop root powder was hydrolyzed by commercial cellulase as described above. Maximum TSS was found at 2.67 ± 0.29 Brix using prop root powder at 100 g/l with 4.0% (v/v) of the commercial enzyme after incubated at 50°C for 24 h, as shown in Figure 2. At low substrate concentrations (25, 50, and 75 g/l), the obtained fermentable sugar was lower than the optimum substrate concentration (100 g/l). TSS increased at higher substrate concentration until beyond the point of optimum concentration [Figure 2a] when TSS slightly decreased due to limits of mixing of enzyme-substrate suspension as reported by Sangngern *et al*. [27] and Lomthong *et al*. [28]. In the case of enzyme concentration, which directly affects the hydrolysis efficiency due to increasing the degradation rate when increasing the enzyme concentration until

Table 2: Average antibacterial area diameter for *S. aureus* DMST 2933 and *E. coli* DMST 4212 of crude pandan prop root extract.

Crude extracted concentration (mg/ml)	Mean zone of inhibition (mm)	
	S. aureus DMST 2933	*E. coli* DMST 4212
100	9.75±0.35^{e}	NA
50	6.75±0.35^{d}	NA
25	5.00±0.71^{c}	NA
12.5	3.00±0.71^{b}	NA
Control	0.00±0.00^{a}	NA

NA is no activity against the growth of bacteria. Different letters within the same column are statistically different at $P<0.05$, *S. aureus*: *Staphylococcus aureus*, *E. coli*: *Escherichia coli*.

the optimum point. Lomthong *et al*. [29] reported that substrate and enzyme concentrations play an important role in sugar syrup production from broken rice. This is similar to Thongpoem *et al*. [30] finding that substrate concentration affected the sugar syrup production from unripe banana flour when using enzymatic hydrolysis. Therefore, the optimum conditions at 100 g/l with 4.0% (v/v) of the commercial enzyme were upscaled in a 5.0 L glass jar chamber to produce pandan prop root syrup and used as a substrate for BC fermentation.

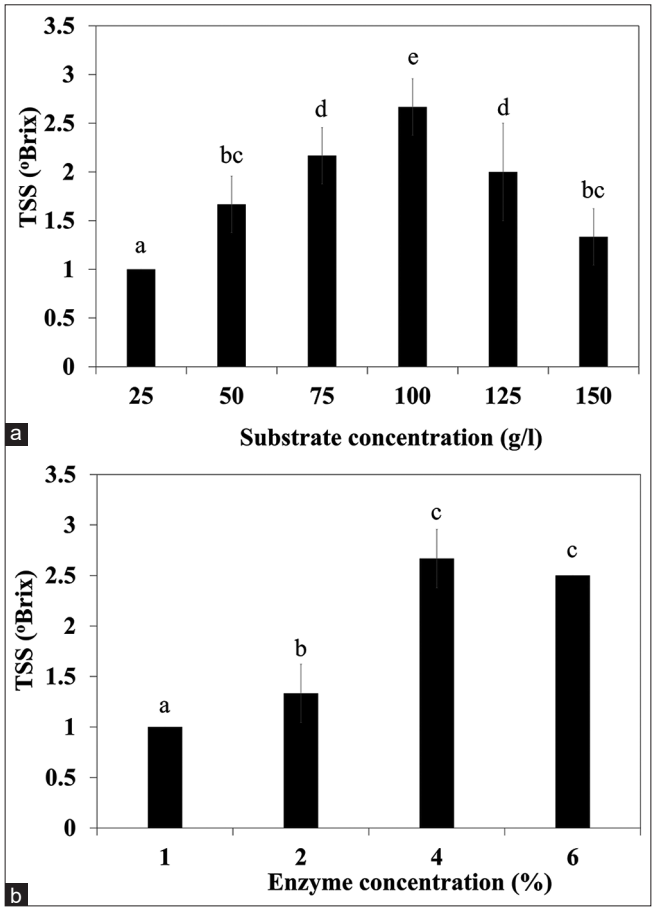

Figure 2: Effects of substrate (a) and enzyme (b) concentrations on total soluble solids (TSS) content from hydrolysis of pandan prop root powder by iKnowZyme® cellulase.

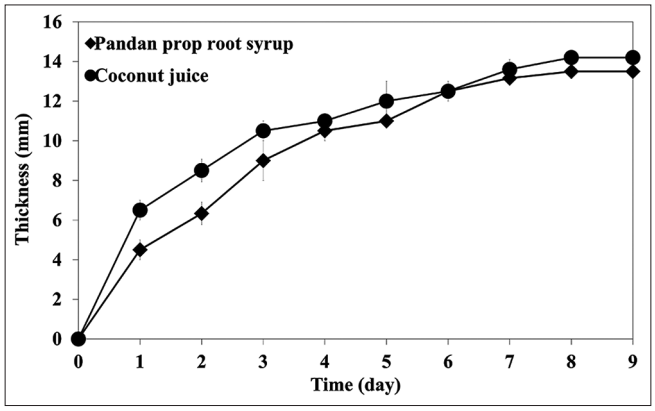

Figure 3: Time course of bacterial cellulose fermentation by *Komagataeibacter xylinus* AGR 60 at room temperature for 9 days.

3.5. Production of BC by *K. xylinus* AGR 60

BC was fermented from pandan prop root syrup using *K. xylinus* AGR 60 at room temperature for nine days. *K. xylinus* AGR 60 was reported as a potent commercial strain for BC production, which coconut juice and other syrups could be used as a substrate for fermentation [13]. Time courses of BC fermentation by pandan prop root syrup and coconut juice are shown in Figure 3. Results indicated that BC produced from pandan prop root had the highest thickness at 13.5 ± 0.05 mm with 7.90 ± 0.10 g of dry weight, and slightly lower than BC produced from coconut juice (14.2 ± 0.60 mm with 8.35 ± 0.22 g). The pH of coconut juice decreased from 4.40 ± 0.10 to 3.43 ± 0.25, while the pH of pandan prop root syrup decreased from 4.37 ± 0.12 to 3.78 ± 0.14. The dry weight matter was used to calculate BC production (P, g/l) and BC production rate (R_p, g/l/day), as shown in Table 3. BC production (P, g/l) values from pandan prop root syrup and coconut juice were 15.80 ± 0.20 and 16.70 ± 0.44 g/l, respectively, while BC production rates were recorded at 1.76 ± 0.02 and 1.85 ± 0.05 g/l/day from pandan prop root syrup and coconut juice, respectively. Moukamnerd *et al*. [12] reported BC production from banana peel and passion fruit peel at 0.89 ± 0.04 and 0.31 ± 0.07 g/l, respectively, and lower than BC production from pandan prop root syrup in this study, while production of BC from pandan prop root syrup was slightly

Table 3: Bacterial cellulose (BC) production parameters from pandan prop root syrup and coconut juice by *K. xylinus* AGR 60.

Parameter	Substrate	
	Pandan prop root syrup	Coconut juice
BC thickness (mm)	13.50±0.05	14.20±0.60
Dry weight (g)	7.90±0.10	8.35±0.22
pH	3.78±0.14	3.43±0.25
BC production (P, g*/l)	15.80±0.20	16.70±0.44
BC production rate (Rp, g*/l/day)	1.76±0.02	1.85±0.05

Values are averages of three determinations, *Based on dry matter, K. xylinus: *Komagataeibacter xylinus*.

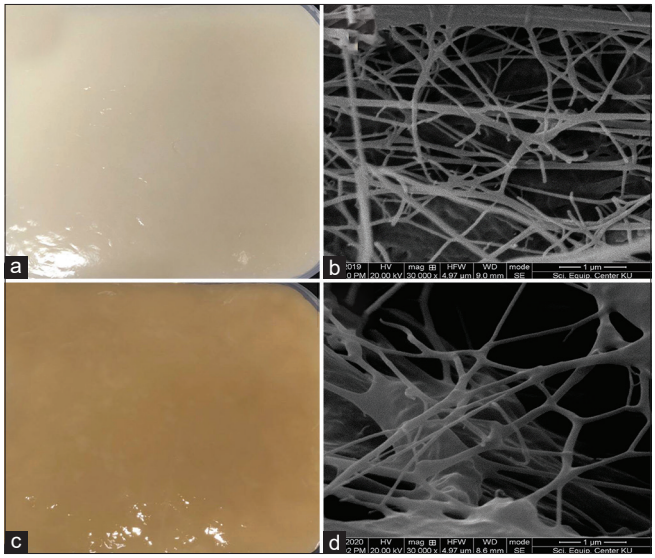

Figure 4: Photographs and scanning electron micrographs of bacterial cellulose produced from coconut juice (a and b) and pandan prop root syrup (c and d) by fermentation of *Komagataeibacter xylinus* AGR 60 at room temperature for 9 days.

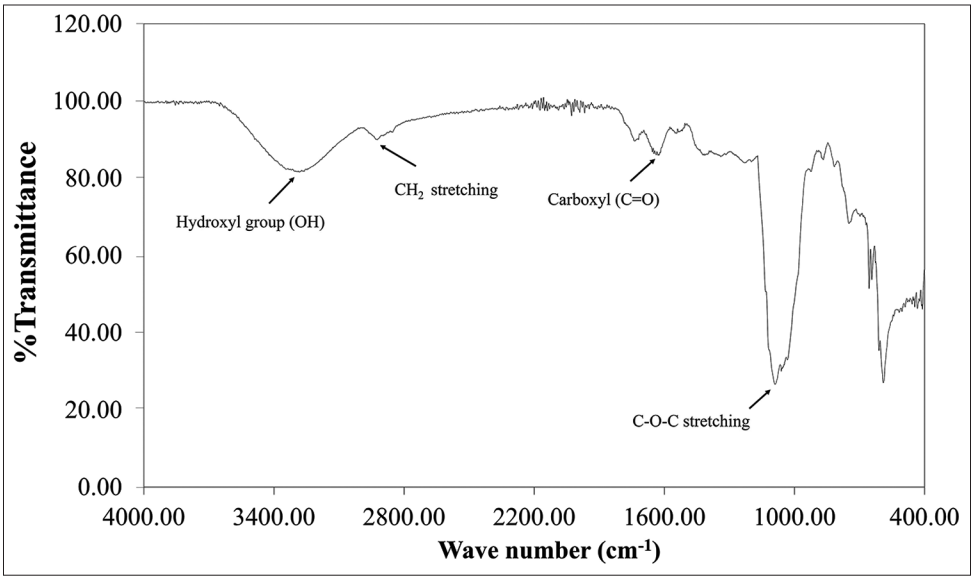

Figure 5: Fourier-transform infrared spectra of the pandan prop root bacterial cellulose at wavenumbers 4000–400 cm^{-1}.

lower than from coconut juice. This study's lower BC production from a novel substrate resulted from some coconut juice nutrients that promoted BC fermentation, such as minerals, vitamins, and amino acids [31]. Noree *et al.* [13] reported lower production of BC from rice syrup than coconut juice by *K. xylinus* AGR 60 that had a similar structure with higher bioactive compound content. Photographs and electron micrographs of BC produced from coconut juice and pandan prop root syrup are shown in Figure 4. The BC sample produced from pandan prop root syrup [Figure 4c] was slightly brown compared to the BC produced from coconut juice [Figure 4a]. The color faded after boiling at 100°C for 30 min. The BC structure investigated by SEM was similar in both samples [Figure 4b and d]. FTIR spectra of BC produced from pandan prop root syrup are shown in Figure 5. FTIR spectra patterns were similar to spectra of BC produced from coconut juice, as reported previously [13]. Bands assigned to –OH and –CH stretching were found at wavenumbers 3200–3400 cm^{-1}, similar to BC produced from banana peel and passion fruit peel reported by Moukamnerd *et al*. [10] and rice syrup reported by Noree *et al.* [13]. The absorption band at 1600–1650 cm^{-1} was recorded as carboxyl (C=O) functional group [32], while peaks at 950–1100 cm^{-1} were recorded as the C–O–C functional group by Noree *et al*. [13]. This study is the first report showing the possibility of using pandan prop root as the substrate to produce novel BC as an alternative application in future functional food and pharmaceutical products.

4. CONCLUSION

P. amaryllifolius Roxb. (Pandanaceae) prop root was shown to be a source of bioactive compounds, with interesting applications for novel healthy foods or functional drink products. Pandan prop root was elucidated for its antioxidant and antibacterial bioactivities to inhibit the growth of *S. aureus*. Application of pandan prop root in BC fermentation using enzymatic hydrolysis was also achieved. Results revealed a novel process and progression in bio-cellulose production technology using enzymatic hydrolysis.

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6. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

7. FUNDING

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8. CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All relevant data are provided within the manuscript.

11. PUBLISHER'S NOTE

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