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Original Research Article

Total Sugar Content and Antioxidant Activity of Nipa Palm (*Nypa fruticans*) Fruit Husk Oligosaccharides

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ABSTRACT

Nipa palm (*Nypa fruticans*) is a monoecious palm widely found in Southeast Asia. The nipa palm fruit husk is a potential source of oligosaccharides that can be used as natural antioxidant agents. This study aimed to extract oligosaccharides from the nipa palm fruit husk, determine their total sugar content, and assess their antioxidant activity. The oligosaccharides were directly extracted using 80% ethanol (EtOH) or a hot water solvent (HW) and then isolated with 80% ethanol. Total sugar content was determined using the phenol-sulfuric acid method and antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The results show that the yield of EtOH (12.58±3.24%) was higher than that of the HW method (7.16±1.03%). The total sugar content of the oligosaccharides was also higher in EtOH (28.84±0.43 mg Glu.eq./g dried sample) compared to the HW method (6.04±0.36 mg Glu.eq./g dried sample). Meanwhile, antioxidant activities were approximately IC_{50} 517.93±92.49 µg/mL (EtOH) and IC_{50} 502.66±17.35 µg/mL (HW). Based on these data, oligosaccharides extracted from nipa palm fruit husks can be used as natural antioxidant sources and formulated as ingredients in food supplements.

Keywords: Antioxidant, extraction, fruit husk, *Nypa fruticans*, oligosaccharides.

Introduction

Nipa palm (*Nypa fruticans*) is a monoecious palm widely found in South Asia, including Indonesia. This plant thrives in brackish water, river estuaries, and mangrove ecosystems where salt and fresh water mix.[1] The chemical composition of the nipa palm includes cellulose, hemicellulose, lignin, starch, protein, and ash. Additionally, the husk of this plant contains several neutral sugars, such as galactose, glucose, mannose, rhamnose, xylose, and arabinose.[2] Based on this information, the authors hypothesized that the nipa palm husk might also contain oligosaccharides. Oligosaccharides are a major group of carbohydrates composed of three to ten monosaccharides, whereas polysaccharides are long-chain carbohydrates.[3] Both oligosaccharides and polysaccharides are formed from monosaccharides linked by glycosidic bonds.[4] Previous studies have reported that oligosaccharides extracted from *Hericium erinaceus*,[5] *Panax ginseng*,[6] and *Evodia lepta*[7] exhibit antioxidant activities. Based on these findings, the authors hypothesized that oligosaccharides from nipa palm husks could also serve as a natural antioxidant source. Antioxidants are substances that help mitigate the harmful effects of free radicals. Oxidative stress occurs when the level of free radicals exceeds the body's antioxidant defenses. In such cases, the body requires external sources of antioxidants, known as exogenous antioxidants.

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These antioxidants include natural bioactive compounds such as oligosaccharides. Oligosaccharides can be extracted from plants using various extraction methods. Previous studies have reported that the hot-water extraction method is effective for extracting polysaccharides, oligosaccharides, monosaccharides, and amino acids from plant materials. Additionally, oligosaccharides can be isolated from other substances due to their solubility in an 80% ethanol solution.[8] Studies have also shown that oligosaccharides, such as fructo-oligosaccharides, galacto-oligosaccharides, and mannan-oligosaccharides, are soluble in 80% ethanol.[9, 10] According to these previous studies that oligosaccharides can be extracted using hot-water or directly using 80% ethanol. However, no study has yet reported the extraction of oligosaccharides from nipa palm husks. Therefore, this study aimed to extract oligosaccharides from the dried husks of nipa palm (*Nypa fruticans*) using either an 80% ethanol solution or a hot-water solvent, followed by isolation with an 80% ethanol solution. Additionally, this study aimed to determine the total sugar content and antioxidant activity of the extracted oligosaccharides.

Materials and Methods

Preparation and extraction

The nipa palm (*Nypa fruticans*) fruit was collected in September 2023 from Sungsang Village, South Sumatra, Indonesia (2.3640° S, 104.8991° E). The fruit was transported to the laboratory, cleaned with water, and the husk was collected and authenticated at the Microbiology and Biotechnology Laboratory of Fisheries Product Technology, Universitas Sriwijaya (FPT0019092023). The husk was then cut into small pieces and dried in an oven at 45°C for 24 hours. After drying, the nipa husk was ground into a powder and stored at cold temperatures for further use. The oligosaccharides from the nipa husk were extracted either directly with 80% ethanol (EtOH) or by hot-water extraction followed by separation with 80% ethanol (HW), with modifications based on previous studies.[11, 12] Briefly, 10 g of dried nipa husk was placed into an Erlenmeyer flask containing 400 mL of EtOH. The extraction was carried out at 90°C, stirred with a magnetic stirrer (IKA hotplate stirrer C-MAG HS 7, IKA Works, Inc., Germany) at 100 rpm for 3 hours. After the extraction, the filtrate and residue were separated using filter paper (Whatman No. 42).

The filtrate was transferred to a new collection tube, and the residue was re-extracted with fresh solvent under the same conditions as the first extraction, for a total of five extractions. The filtrates were combined, and the solvent was evaporated before drying in an oven at 70°C to obtain the dried extract powder (EtOH extract). For the hot-water extraction, 10 g of dried nipa husk was extracted with 400 mL of distilled water at 90°C, stirred with a magnetic stirrer at 100 rpm for 3 hours. The supernatant (filtrate) and residue were separated by centrifugation (Oregon centrifuge LC-04C Plus, Oregon Scientific, Hong Kong) at 4,000 rpm for 20 minutes. The filtrate was transferred to a new collection tube, and the residue was re-extracted with fresh solvent under the same conditions, for a total of five extractions. The filtrates were combined, and 80% ethanol was added to precipitate the oligosaccharides. The supernatant was collected by centrifugation at 4,000 rpm for 10 minutes, then dried in an oven at 70°C to obtain the dried extract powder (HW extract. The yield of extraction was calculated according to Equation 1:

$$\text{Yield (\%)} = \frac{\text{Extract weight (g)}}{\text{Dried sample weight (g)}} \times 100\% \quad \text{Equation 1}$$

Total sugar analysis

The total sugar content of the oligosaccharide extract was determined using the Phenol-Sulfuric acid method, as described in the previous protocol.[13] Briefly, 10 mL of oligosaccharide solution (5 mg/mL in 80% ethanol) was transferred into a reaction tube. The reaction was conducted at 90°C in a water bath for 10 minutes, then allowed to cool to room temperature. Next, 1 mL of the solution was combined with 0.5 mL of 5% phenol (phenol in water, v/v). The mixture was then treated with 2.5 mL of 36 N sulfuric acid and incubated for 20 minutes at room temperature. Absorbance was measured immediately at 490 nm using a UV-Vis spectrophotometer (Genesys 150, ThermoScientific, Thermo Fisher Scientific, USA). Glucose was used as the standard for calculating the total sugar content. The total sugar of the oligosaccharide was expressed as mg glucose equivalent per gram of dried sample (mg Glu.eq./g dried sample).

Antioxidant activity assay

The antioxidant activity of the oligosaccharide was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, as described in previous studies.[14-16] Briefly, the oligosaccharide extract was dissolved in ethanol to create a series of concentrations (0 – 1000 µg/mL). Then, 1 mL of each sample solution was mixed with 1 mL of 0.2 mM DPPH solution (DPPH dissolved in methanol) and incubated at 37°C for 30 minutes. After incubation, the absorbance was measured immediately at 517 nm using a UV-Vis spectrophotometer. The antioxidant activity was expressed as the percentage inhibition of DPPH free radicals by the sample solution, calculated using Equation 2:

$$\text{Percentage inhibition (\%)} = \frac{Abs_{\text{blank}} - Abs_{\text{sample}}}{Abs_{\text{blank}}} \times 100\% \quad \text{Equation 2}$$

Whereas: Abs_{blank}, absorbance at 517 nm without sample, Abs_{sample}, absorbance at 517 nm with sample.

FT-IR analysis

The functional groups, including the glycosidic linkage and hydroxyl groups, of the oligosaccharide were identified using Fourier-transform infrared (FT-IR) spectroscopy (InfraRed Bruker Tensor 37, Bruker Corporation, USA), following the method described in previous studies.[17] The FT-IR spectra of the oligosaccharide were recorded by mixing the sample with potassium bromide and pressing the mixture into pellets.

Statistical analysis

All data are presented as the mean ± standard deviation (SD) and analyzed using an independent samples t-test. Statistical significance was set at $p<0.05$, and the analysis was performed with SPSS software (v.22.0; IBM Corp., NY, USA).

Results and Discussion

Yield of extraction

The oligosaccharides were successfully extracted from nipa palm fruit husk using either the EtOH or HW extraction methods. The yields of oligosaccharide extraction are shown in Figure 1. The yields of EtOH and HW oligosaccharides were approximately 12.58±3.24% and 7.16±1.03%, respectively. These results indicate that the yield from EtOH extraction is significantly ($p<0.05$) higher than that from the HW extraction. This suggests that the EtOH extraction method yields a higher content of crude oligosaccharides compared to the HW metho. A previous study reported that oligosaccharides can be extracted from plant parts or isolated from other substances using an 80% ethanol solution. The same study found that approximately 1.05% of crude oligosaccharides were successfully extracted from red yeast rice.[11] Additionally, oligosaccharides can also be extracted using hot water; however, this extract is typically composed of more complex polysaccharides.[12] Therefore, the oligosaccharides were isolated from these complex polysaccharides using 80% ethanol. Previous studies have shown that oligosaccharides are soluble in 80% ethanol,[10] whereas polysaccharides tend to precipitate in 80% and 96% ethanol solutions.[18, 19] Furthermore, ethanol, including 80% ethanol, is commonly used for polyphenol extraction,[20] as polyphenols are less soluble in water.[15] Thus, the higher extraction yield from EtOH may also include crude polyphenol extracts, which explains why the EtOH extraction method yields more oligosaccharides than the HW method.

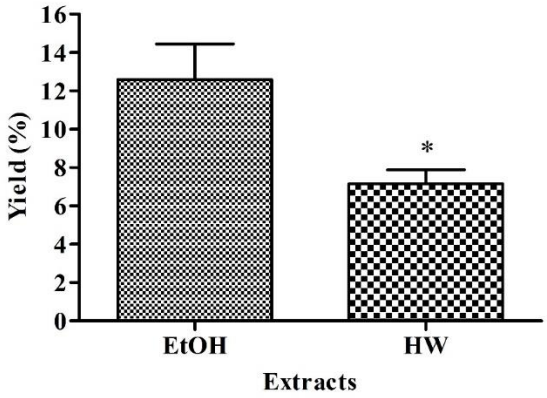

Figure 1. The yield of oligosaccharides from nipa palm (*Nypa fruticans*) fruit husk with different extraction methods. The data are shown as the mean ± SD (n=3). Statistical significance at *$p<0.05$ versus EtOH. EtOH, oligosaccharides are directly extracted by using 80% ethanol; HW, oligosaccharides extracted by using hot-water then isolated with 80% ethanol.

Total sugar contents

The total sugar content of the oligosaccharide extract is shown in Figure 2. The total sugar content of the oligosaccharides extracted using EtOH (28.84±0.43 mg Glu.eq./g dried sample) was significantly ($p < 0.05$) higher than that obtained with the HW method (6.04±0.36 mg Glu.eq./g dried sample). The total sugar content reflects the number of reducing sugars, such as lactose, fructose, and glucose as well as oligosaccharides that can be hydrolyzed into reducing sugars under the measurement conditions.[21] The total sugar was analyzed using the Phenol-Sulfuric acid method, which is widely employed due to its high sensitivity, good stability, simple detection process, and lack of interference from proteins.[21, 22] A higher sugar content was detected in the EtOH extracts. Previous studies reported that 80% ethanol can also be used to extract polyphenolic compounds, which are often conjugated with sugar, rather than being free.[23, 24] Therefore, the use of 80% ethanol enhances the total sugar content compared to the HW extraction method, likely due to the lower solubility of polyphenolic compounds in water.[15, 25]

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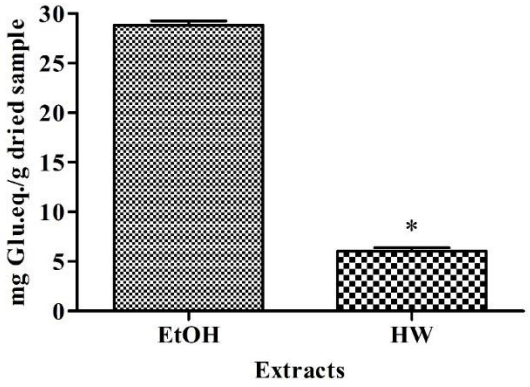

Figure 2. The total sugar content of oligosaccharides from nipa palm (*Nypa fruticans*) fruit husk extract with different extraction methods. The data are shown as the mean ± SD (n=3). Statistical significance at *p<0.05 versus EtOH. EtOH, oligosaccharides are directly extracted by using 80% ethanol; HW, oligosaccharides extracted by using hot-water then isolated with 80% ethanol.

Antioxidant activity

The antioxidant activity of oligosaccharides from nipa palm fruit husk is shown in Figure 3. There was no significant difference (p>0.05) in antioxidant activity between the two extraction methods. The IC_{50} value for EtOH extraction was approximately 517.93±92.49 µg/mL, while that for HW extraction was 502.66±17.35 µg/mL. Antioxidant activity was analyzed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, which is favored for its simplicity, rapidity, and low cost.[26] A previous study also reported that oligosaccharides isolated from *Evodia lepta* exhibit antioxidant activity (IC_{50} 110 µg/mL – 170 µg/mL) using the DPPH method.[7] The antioxidant activity was measured based on the ability of the compounds to donate a hydrogen (H) atom to the DPPH radical.[27] Thus, the oligosaccharides from nipa palm husk may act as antioxidants by donating hydrogen to free radicals. Oligosaccharides are a major class of carbohydrates consisting of three to ten monosaccharide units.[3] A previous study reported that carbohydrates scavenge OH radicals through a hydrogen atom transfer mechanism.[28]

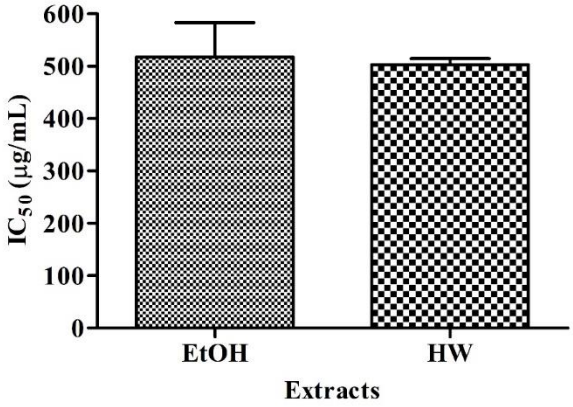

Figure 3. The antioxidant activity of oligosaccharides from nipa palm (*Nypa fruticans*) fruit husk extract with different extraction methods. The data are shown as the mean ± SD (n=3). EtOH, oligosaccharides are directly extracted by using 80% ethanol; HW, oligosaccharides extracted by using hot-water then isolated with 80% ethanol.

Functional groups of the nipa palm husk oligosaccharide

The functional groups of the oligosaccharide extract from nipa palm husk are shown in Figure 4. The O–H stretching vibration was detected at 3502.77 cm^{-1} (EtOH) and 3347.12 cm^{-1} (HW), while the glycosidic C–O–C linkage was observed at 1103.18 cm^{-1} (EtOH) and 1080.26 cm^{-1} (HW). The carbonyl (C=O) stretching appeared at 1618.72 cm^{-1} (EtOH) and 1621.50 cm^{-1} (HW). The pyran-type sugar ring was detected at 530.96 cm^{-1} (EtOH) and 535.20 cm^{-1} (HW). These functional groups were analyzed according to previous studies.[29, 30] Additionally, the peaks in the range of 950–1200 cm^{-1} were attributed to the stretching vibrations of the C–C and C–O bonds in the pyran ring.[30] The water bound to syringyl-derived compounds or to the sugar chain linked to oligosaccharides was identified by the band at 1604 cm^{-1}.[31] Oligosaccharides are a major class of carbohydrates that consist of three to ten monosaccharide units, whereas polysaccharides are carbohydrates made up of long-chain monosaccharides linked by glycosidic bonds.[3] Both oligosaccharides and polysaccharides are composed of monosaccharides connected through glycosidic bonds.[5, 32] A previous study reported the following functional groups in *Acacia tortilis* polysaccharides: glycosidic linkage (1030.5 cm^{-1}), carbonyl stretching (1630.4 cm^{-1}), C–H stretching (2940.3 cm^{-1}), and O–H stretching (3430.6 cm^{-1}).[17]

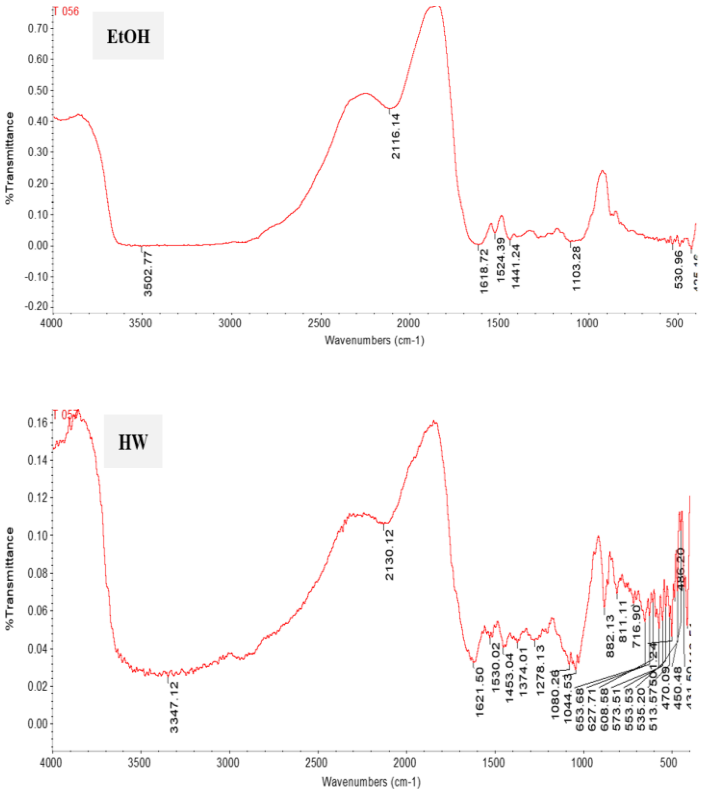

Figure 4. The FT-IR spectra of oligosaccharide extracts from nipa palm (*Nypa fruticans*) fruit husk. EtOH, oligosaccharides are directly extracted by using 80% ethanol; HW, oligosaccharides extracted by using hot-water then isolated with 80% ethanol.

Conclusion

Overall, oligosaccharides were successfully extracted and isolated from nipa palm (*Nypa fruticans*) fruit husks. Extraction with an 80% ethanol solution resulted in a high yield of oligosaccharides and a significant sugar content. Additionally, the oligosaccharides exhibited antioxidant activity. Therefore, oligosaccharides from nipa palm fruit husk could serve as a natural source of antioxidants and may be formulated as a potential food supplement.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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