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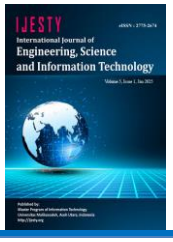

Isolation and Fractionation of Lignin from Sugar Cane Bagasse (Saccharum Officinarum L.) as an Antioxidant

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Abstract

Bagasse is one of the solid wastes of the sugar cane industry, and it contains lignin fibre. Lignin from sugarcane bagasse is also known to have relatively high antioxidant activity. Sugarcane bagasse antioxidants can stabilize free radicals by completing free radicals' lack of electrons and inhibiting chain reactions from forming free radicals. This type of research is laboratory experimental research (actual experiment design). In this research, lignin isolation and fractionation will be carried out from sugarcane bagasse to obtain lignin fractions with antioxidant activity. The percentage of lignin yield from this process is around 72.85%. The IR spectrum resulting from isolation from sugarcane bagasse has a typical absorption peak of aliphatic and aromatic –CH at a wave number of 2919.7/cm. The antioxidant activity of the lignin fraction from sugarcane bagasse was determined using the DPPH (1,1-diphenyl-2- picrylhydrazyl) free radical scavenging method using four solvents, namely ethyl acetate, acetone and methanol. Based on the level of antioxidant strength, the strength level is <50 powerful, 50-100 strong, 100-250 medium, 250-500 weak and >500 inactive; therefore, the results of testing the most potent antioxidant activity in the acetone fraction were found to be $IC50$ 50, 6755 mg/L and weak antioxidant activity was found in the methanol fraction, the result was $IC50$ 68.8503.

Keywords: *Bagasse, Lignin, Antioxidant, DPPH, Isolation.*

1. Introduction

Sugarcane (Saccharum officinarum L.) is an essential economic crop in many countries, including Indonesia, and is primarily used for sugar production. After extracting the juice from sugarcane, the by-product is called bagasse, a fibrous waste. Bagasse has limited applications, mainly in composite materials, textiles, pulp and paper production, animal feed, and as fuel in sugar industries. Approximately 90% of the sugarcane bagasse is produced as a residue during sugar production.

Sugarcane bagasse contains lignin, a component of plant cell walls, particularly in woody plants and fiber-producing plants like sugarcane. Lignin accounts for 22-30% of bagasse's composition and has a high antioxidant and antimicrobial activity. It consists of phenylpropane units, including syringyl (S), guaiacyl (G), and p-hydroxyphenyl (H) units. Lignin is low in toxicity, biocompatible, and abundant in nature. It is also a potential raw material for various industrial and health applications. The antioxidant activity of lignin is influenced by factors such as molecular weight, molecular weight distribution, and phenolic group content.

This study isolates and fractionates lignin from sugarcane bagasse to identify fractions with significant antioxidant activity, determined by the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging method.

2. Methods

This study employs a laboratory experiment (actual experiment design) to isolate and fractionate lignin from bagasse and test its antioxidant activity.

Materials: Sugarcane bagasse, ethanol, aquadest, NaOH (3 M), H2SO4, ethyl acetate, acetone, methanol, DPPH, and BHT.

Equipment: Grinder, mesh sieve (40 mesh), analytical balance, reflux apparatus, water bath, oven, spectrophotometer UV-Vis, and FTIR spectrometer.
The procedural steps are:

a. Sample Preparation
Bagasse was cleaned, dried in an oven, and ground into fine powder using a grinder. The powder was sieved with a 40-mesh sieve to ensure uniform particle size.

b. Lignin Isolation
1. Reflux Process: 20 g of bagasse powder was subjected to sequential refluxing using 250 mL of ethanol (8 hours, 120°C), aquadest (2 hours, 80°C), and 250 mL of NaOH 3 M (4 hours, 40°C).
2. Filtration and Precipitation: The filtrate was acidified with H2SO4 to pH 2, leading to lignin precipitation. The lignin was filtered, washed with 0.1 M HCl, and dried at 70°C.
3. Characterization: Lignin's functional groups were identified using FTIR spectroscopy.

c. Lignin Fractionation
The lignin was fractionated using ethyl acetate, acetone, and methanol. Each fraction was collected, evaporated, and labelled as F1 (ethyl acetate), F2 (acetone), F3 (methanol), and F4 (residue).

d. Antioxidant Activity Test (DPPH Method)
1. Preparation of DPPH Solution: 0.2 mM DPPH solution was prepared in methanol.
2. Sample Preparation: Lignin fractions (F1-F4) were prepared at concentrations of 3, 6, 12, 25, and 50 mg/L.
3. Measurement: Each concentration was mixed with DPPH solution, and the absorbance was measured at 516 nm using a UV-Vis spectrophotometer.
4. Data Analysis: The percentage of radical scavenging activity was calculated, and the IC50 value was determined using a linear regression equation.

3. Results and Discussion

3.1. Sugarcane Bagasse

Plant determination for this study was conducted at BRIN (National Research and Innovation Agency) Cibinong, Bogor. The results showed that the sample used was Sugarcane (Saccharum officinarum L.), with Number B- 1916 / II.6.2 / IR.01.02 / 8/2023. The determination results can be seen in Appendix 1.

Fig 1. The results obtained from the preparation of sugarcane bagasse

3.2. Isolation, Fractionation, and Characterization of Lignin

Isolation yield: $\frac{2{,}22g}{20} \times 100\% = 11{,}1\%$

Table 1. Fractionation of bagasse lignin based on solvent

Results	**Initial weight (g)**	**Final weight (g)**	**Yield (%)**
Sugarcane ethyl acetate extract	3	0,0902	3,006%
Sugarcane acetone extract	3	0,2485	8,2833%
Sugarcane methanol extract	3	0,2883	9,61%
Sugarcane F4 extract	3	2,1856	72,853%

1. Lignin Yield:
The yield of lignin isolation is 72.85%, meaning that 72.85% of the total solid mass obtained after evaporation consists of lignin. This indicates an effective isolation process.
2. Solid Mass:
The highest solid mass was obtained from isolation and fractionation using residual solvent (F4), while the lowest was from ethyl acetate solvent.

3. IR Spectrum Analysis:
   a. The IR spectrum of lignin from sugarcane bagasse shows characteristic stretching peaks of aliphatic and aromatic –CH at a wavenumber of 2919.7 cm^{-1}, similar to commercial lignin (Aldrich: 2930.17 cm^{-1}, Kraft: 2926.01 cm^{-1}).
   b. The stretching vibration of the –C=C– aromatic functional group appears at 1511.92 cm^{-1} for sugarcane bagasse lignin, while for Aldrich lignin, it is at 1599.14 cm^{-1} and for Kraft lignin at 1614.42 cm^{-1}.

3.3. Antioxidant Testing with DPPH Method

Data obtained from absorbance at various concentrations were used to calculate antioxidant activity and compare the effectiveness of multiple samples as follows:

Table 2. Results of antioxidant testing of sugarcane bagasse lignin using the DPPH method

Fraction	Concentration (mg/L)	Average Absorbance (516 mm)	Inhibition (%)	IC_{50} (mg/L)
Ethyl acetate	3	0,879515	23,39	55,9046
	6	0,853264	25,68	
	12	0,821811	28,41	
	25	0,751724	34,52	
	50	0,607362	47,09	
Aceton	3	0,866845	24,49	50,6755
	6	0,836846	27,11	
	12	0,776805	32,33	
	25	0,691772	39,74	
	50	0,594049	48,25	
Methanol	3	0,869283	24,28	68,8503
	6	0,839751	26,85	
	12	0,824773	28,16	
	25	0,761675	33,65	
	50	0,658089	42,68	
F4	3	0,868904	24,31	62,5011
	6	0,837621	27,04	
	12	0,813127	29,17	
	25	0,752601	34,44	
	50	0,635529	44,64	
BHT	3	0,69527	39,44	13,3166
	6	0,66835	41,78	
	9	0,61792	46,17	
	12	0,59108	48,51	
	15	0,55502	51,65	

1. Inhibition Percentage:
   The highest is in acetone and the lowest is in methanol, and it increases with solvent volume.
2. Antioxidant Activity:
   Ethyl Acetate: 23.39% to 47.09% (3-50 ppm)
   Acetone: 24.49% to 48.25% (3-50 ppm)
   Methanol: 24.28% to 42.68% (3-50 ppm)
   F4: 24.31% to 44.64% (3-50 ppm)
3. BHT:
   Most muscular inhibition (39.44% to 51.65%, 3-15 ppm) with the lowest IC50 (13.3166 mg/L).
4. IC50:
   BHT had the most potent inhibition, with higher IC50 values for Ethyl Acetate, Methanol, F4, and Acetone.

3.4. Discussion

1. Research Phases:
   The study involves four stages: preparation of sugarcane bagasse (Saccharum officinarum L.), lignin isolation, lignin fractionation, and antioxidant testing using the DPPH method.
2. Sample Preparation:
   Sugarcane bagasse was sorted, cleaned, and chopped. The cleaning process removed unwanted debris, ensuring a pure sample for extraction. The chopped bagasse was then dried to reduce moisture content, which could lead to spoilage or microbial growth. After drying, the bagasse was ground to a fine powder, ensuring uniform particle size for efficient extraction.

3. Lignin Isolation:
   Lignin was isolated using 20g of dried, ground sugarcane bagasse. The isolation involved refluxing the bagasse with ethanol, equates, and NaOH to remove non-polar and polar components. NaOH was used to break the bonds between lignin and other elements, resulting in a brown filtrate containing dissolved lignin. The lignin was then precipitated using concentrated H_2SO_4 and dried to produce lignin powder.
4. Lignin Characterization:
   The isolated lignin was analyzed using FTIR spectroscopy. The results showed that the lignin extracted from sugarcane bagasse had functional groups similar to those found in commercial lignins, such as aromatic -CH and -C=C groups and phenolic -OH groups.
5. Lignin Fractionation:
   Lignin was fractionated using ethyl acetate, acetone, and methanol to isolate different lignin fractions based on solubility. The fractions showed varying polarities and chemical properties.
6. Antioxidant Testing:
   Antioxidant activity was assessed using the DPPH method. The results showed that the ethyl acetate and acetone fractions had significant antioxidant potential, with increasing inhibition percentages at higher concentrations. The methanol and residual fractions (F4) also showed antioxidant activity but to a lesser extent. BHT, a synthetic antioxidant, showed the highest activity compared to all lignin fractions, confirming its efficiency as a control.

4. Conclusion

1. Lignin Fraction Characteristics
   a. Yield percentages of sugarcane bagasse lignin fractions:
   b. Ethyl Acetate: 3.00%
   c. Acetone: 8.28%
   d. Methanol: 9.61%
   e. Residual: 72.85%
   f. The IR spectrum of isolated lignin shows characteristic absorption peaks:
   g. Aliphatic and aromatic –CH stretching at 2919.7 cm^{-1}.
   h. –C=C– a functional group of aromatic rings at 1511.92 cm^{-1}.
2. Antioxidant Activity
   a. Ethyl Acetate Fraction: Inhibition increases from 24.49% (3 ppm) to 48.25% (50 ppm) with an IC50 of 55.90 mg/L.
   b. Acetone Fraction: Inhibition increases from 24.49% (3 ppm) to 48.25% (50 ppm) with an IC50 of 50.57 mg/L.
   c. Methanol Fraction: Inhibition increases from 24.28% (3 ppm) to 42.68% (50 ppm) with an IC50 of 68.85 mg/L.
   d. F4 Fraction: Inhibition increases from 24.31% (3 ppm) to 44.64% (50 ppm) with an IC50 of 62.50 mg/L.
   e. BHT (Control): Shows the highest antioxidant activity with inhibition increasing from 39.44% (3 ppm) to 51.65% (15 ppm) and IC50 of 13.31 mg/L.

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