Antioxidant Activity, Total Phenolic Content, and Chemical Composition of Liquid Smoke Derived from Wood Sawdust

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ABSTRACT
Liquid smokes result from the process of pyrolysis of biomass as a raw material which generally contains high content of lignin, cellulose, and hemicellulose. The phenolic compounds, acid compounds, and carbonyl compounds are the main components in liquid smoke and show antioxidant activity. One of the potential raw materials from industrial's waste that has potentiality as liquid smoke is wood sawdust. Phenolic compounds that can be found as a prevalent composition in wood sawdust have antioxidant activity and it is potential to be used as a material for food preservation to maintain freshness and extend product shelf life. The manufacture of liquid smoke from biomass is carried out through several stages, starting from burning biomass at high temperatures (pyrolysis), condensation, and ending up with purification. The purification stage aims to remove toxic compounds resulting in purified products. The present study aimed to evaluate the crude and purified liquid smoke derived from wood sawdust and its phenol composition. Investigation of phenol compound was conducted by Folin-Ciocalteu method and Gass-Chromatography Mass Spectroscopy for further analysis. The crude and purified liquid smoke's antioxidant presented 46.24 µg/ml and 1404.89 µg/ml respectively. Regarding the total phenolic content, the phenol content in crude liquid smoke was 95.93 µgGAE ± 1.15 µgGAE whereas the phenol content on purified liquid smoke was 39.27 ± 2.67 µgGAE. Finally, there were approximately seven phenolic compounds observed in gas chromatography-mass spectrometry analysis. Among the detected compound, Phenol (CAS) Izal and Phenol, 2- methoxy- (CAS) Guaiacol were found as dominant phenolic compounds both in crude and purified liquid smoke. This study proved that high phenolic compounds in crude liquid smoke had the effectiveness for observed antioxidant properties against free radicals.

Keywords: *ABTS, GC-MS, Liquid smoke, TPC, Wood sawdust.*

1. INTRODUCTION

Liquid smoke is produced from the pyrolysis of biomass and then condensation which generally contains high levels of lignin, cellulose, and hemicelluloses [1]. Most of the biomass used as raw material for pyrolysis is obtained from woodresidues in several waste products ranging from agriculture, forestry, to the wood industry. Wood sawdustis one of the wood waste products that has not been optimized effectively, although, the waste of wood sawdust contains several macromolecules such as lignin,cellulose and hemicellulose which can be used as raw material for liquid smoke. The content of lignin, cellulose, and hemicelluloses differs depending on the type of wood, wood moisture, and temperature of the pyrolysis process. Therefore, the physical andchemical properties of each wood sawdust are different [2].

The manufacture of liquid smoke from biomass is carried out through several stages, starting from burning biomass at high temperatures (pyrolysis), condensation, and ending up with purification. Pyrolysis is the process of decomposition of a material at high temperatures that takes place in the absence of air or confined air. Pyrolysis consists of several stages. The initial stage commences with the evaporation of the water, next to the breakdown of hemicellulose, cellulose, and lignin [1]. The purification stage aims to remove toxic compounds. Liquid smoke pyrolysisproducts need to be purified such as adsorption anddistillation to increase liquid smoke products. An active zeolite is used in the adsorption process because it has high selective cation exchange capacity (CEC), which makes itsuitable for removing impurities [3]. After the adsorption stage, the liquid smoke remains black, therefore a distillation process is required to clean the liquid smoke as a function of the difference in the boiling points. The distillation process serves to remove tar which is a Polycyclic Aromatic Hydrocarbons (PAH). Polycyclic Aromatic Hydrocarbon (PAH) must be removed due to their carcinogenic and mutagenic characteristics [4].

There are some differences in the crude and purified liquid smoke after the distillation process including no more tar, clearer liquid smoke after distillation, lowerpH resulting from distillation, a higher concentration of acetate acid after distillation, and neutral aroma after the distillation process [5]. Similar results were also obtained by Fauzan and Ikhwanus, 2017 [6] that with the distillation and filtration process liquid smoke has different characteristics from crude, namely clear colour, slightly sour taste, neutral aroma, and does not contain harmful compounds. Therefore, this study aims to determine the antioxidant activity and total phenols of crude and purified liquid smoke derived from wood sawdust.

2. METHODS

2.1 Pyrolysis

The pyrolysis of wood sawdust biomass was conducted by this process parameters; wood sawdust weighing 7.4 kg was heated to 530 ^{o}C for 8 h in a pyrolysis reactor. The temperature of 530 ^{o}C was chosen as an appropriate combustion temperature that can be used to pyrolyze the wood sawdust [5]. The condensed pyrolysis product in the form of liquid smoke is placed in a container and incubated for 24 h to precipitate the tar.

2.2 Purification

The natural materials used as adsorbents for purification are zeolite and activated carbon. Purification process was carried out at the Balai Penelitian Teknologi Bahan Alam (BPTBA), Badan Riset dan Inovasi Nasional (BRIN), Gunung Kidul, Yogyakarta Special Region. Purification was carried out by soaking 50 ml of a liquid smoke sample using 2 g of adsorbent (carbon: zeolite = 1:1) for 3 h.

2.3 ABTS Assay

The antioxidant activity of liquid smoke was determined by ABTS 2,2′-Azino-bis (3-ethyl benzthiazoline-6-sulfonic acid) according to the method of [7] A 7.4 mM ABTS solution was prepared by dissolving 40.6 mg ABTS (Sigma) in 10 ml of distilled water. Potassium peroxydisulfate 2.6 mM solution was prepared by dissolving 7.03 mg of the material into 10 ml of distilled water. ABTS solution and Potassium peroxydisulfate solution in a ratio of 1: 1 were mixed in a dark bottle. The reagent mixture was incubated for 16 hours at room temperature and protected from light exposure. Tests were carried out using 96 well plates (Nunc). Each of the samples prepared stock solution with a concentration of 1000 µg/mL (as the first concentration).Then the stock solution was diluted in a serial concentration of 625 µg/mL, 312.5 µg/mL, 156.25 µg/mL, 78.125 µg/mL, and 39.06 µg/mL. In each well 15 µl of the sample was put in and then added by 285 µl of 7.4 mM ABTS solution. Amounts of 15 µl distilled water were added with 285 µl of 7.4 mM ABTS solution used as blank. The mixture was incubated for 120 min in a dark place. Elisa reader (Multiskan™ FC Microplate Photometer) at a wavelength of 734 nm was used to measure the absorbance. For comparison, ascorbic acid (Merck) was used. Data analysis used ANOVA with SPSS (Statistical Software, InC., Chicago, IL, USA) package.

2.4 Total Phenolic Content (TPC) with Folin-Ciocalteu

Folin-Ciocalteu assay with modification [8] was used to determine the total phenol content of samples with gallic acid as a standard. Firstly, 0.25 mL of liquid smoke (1000 µg/ml) was taken and mixed with 3.75 ml distilled water, then 0.25 ml of Folin- Ciocalteu reagent was added. After that, the mixture was vortexed for 1 min and incubate for 8 min. The mixture was added 0.75 ml of Na_2CO_3 (20%) and incubation at room temperature for 120 min. Then, the absorbance was measured at a wavelength of 765 nm. The same application is also carried out on gallic acid as astandard solution. The total phenolic content was expressed as µg of gallic acid equivalent.

2.5 Chemical Composition with GC-MS

Samples of liquid smoke were analyzed for acidity with pH meter (Mettler Toledo) and chemical composition using a GC-MS (GC-2010 / QP2010S, Shimadzu) with a DB-624 column (Agilent Technologies, Inc. 30 m × 250 µm × 1.40 µm). For chemical composition analysis, helium is used as a carrier gas. The temperature used in the injectoris 250 °C. The initial temperature of the column in the oven was 40 °C (maintained 5 min), raised to 190 °C at 4 °C/min, and the final temperature at 190 °C (maintained 17.5 min).

Furthermore, 240 °C were set up for source and interface temperature. Ionization energy was 70 eV and the mass range was m/z 28 AMU to 600 AMU respectively. The total flow, column flow, and linear velocity were 36 mL/min, 0.86 mL/min, 33.2 cm/s respectively. Next, the chromatograms and MS data obtained were downloaded and further analysed.

3. RESULTS

3.1 Antioxidant Activity

Among the sample, both crude and purified liquid smoke possess different ABTS-reducing activities. Crude liquid smoke had the highest ABTS-reducing activity compared to purified liquid smoke (Table 1). This result assured that crude liquid smoke exhibited effective antioxidant activity while purified liquid smoke has a high IC_{50} value which indicates this compound is noteffective in reducing the ABTS radical.

3.2 Total Phenolic Content

Figure 1 shows the difference in phenolic content of crude and purified liquid smoke and their standards. The total phenolic content was measured using GAE as a standard. The highest total phenolic contents were found in crude liquid smoke (95.93 µgGAE ± 1.15 µgGAE) that is followed by purified liquid smoke (39.27± 2.67 µgGAE).

3.3 Chemical Composition

The GC-MS was performed to identify the potent phenol compound that is mainly found in liquid smokes

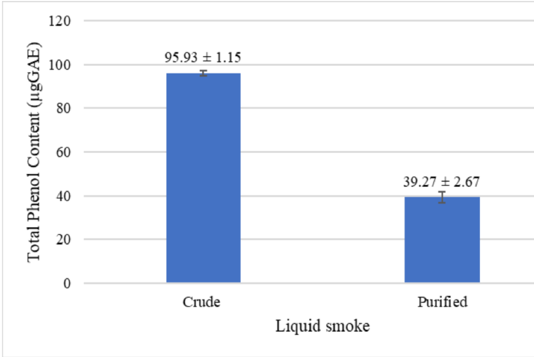

Figure 1. Phenolic content of liquid smoke derived from wood sawdust. (The data was presented as mean ± standard deviation)

and can be attributable as an antioxidant. It can be shown in Table 2, seven phenol compounds were detected in crudeand purified liquid smoke. The dominant peak at 4.71 % and 4.22 % showed the presence of phenol compounds namely Phenol (CAS) Izal and Phenol, 2-methoxy- (CAS) Guaiacol.

4. DISCUSSIONS

The antioxidant activity of a compound can be determined using several techniques. Unstable free radical molecules are known to play a definite role in various pathological manifestations. Free radicals can be countered with antioxidants by producing macromolecular protection. Antioxidants are stable molecules that have the ability to combat free radicals by donating electrons to the raging free radicals and neutralizing them, thereby reducing their capacity to damage other molecules. Liquid smoke as the result of condensation or vapour condensation of wood residues

Table 1. ABTS activity of liquid smoke and ascorbic acid

Sample	Concentration (µg/mL)	ABTS-reducing activity (%)	IC50 (µg/ml)
Crude liquid smoke	625	88.70 ± 1.36	46.24[a]
	312.5	74.46 ± 3.45	
	156.25	59.63 ± 6.84	
	78.13	54.64 ± 0.54	
	39.06	43.11 ± 4.57	
Purified liquid smoke	625	26.40 ± 1.57	1404.89[b]
	312.5	17.98 ± 0.27	
	156.25	12.88 ± 2.31	
	78.13	10.01 ± 2.08	
	39.06	8.97 ± 2.89	
Ascorbic acid	125	94.53 ± 0.60	52.59[a]
	61.25	73.25 ± 6.46	
	30.63	32.87 ± 3.36	
	7.66	13.85 ± 3.47	
	3.83	10.56 ± 4.32	

*All values are mean ± SD of 3 replicates and all the experiments were repeated three times.Different lower case (a-b) in the same column indicates significant difference ($p<0.05$).

Table 2. The results of e chromatogram mass spectrum analysis of phenol composition of liquid smoke

No	Name of compound	Area (%)		Activity	References
		Crude liquid smoke	Purified liquid smoke		
1	Phenol (CAS) Izal	4.71	4.22	Antioxidant, Antibacterial	[9] [10]
2	Phenol, 2,3-dimethyl-	0.26	-	Antioxidant, fragrant aroma	[11] [12]
3	Phenol, 2,3-dimethyl- (CAS) 2,3-Dimethylphenol	-	0.17	Antioxidant, fragrant aroma	[11] [12]
4	Phenol, 2-methoxy- (CAS) Guaiacol	4.71	4.22	Antioxidant, Antibacterial	[9] [10]
5	Phenol, 2-methoxy-4-methyl-	0.77	0.41	Antibacterial	[13]
6	Phenol, 4-ethyl-2-methoxy- (CAS) p-Ethyl guaiacol	0.4	0.14	Antifungal	[14]
7	Phenol, 3-ethyl-	-	0.25	Antibacterial	[15]
8	Phenol, 3-methyl-	1.92	1.09	No activity	[16]

from the process of pyrolysis reported has antioxidant activity.

ABTS assay can measure the electron donation ability of liquid smoke. ABTS radical has a characteristic of solubility in both aqueous and organic solvents. Therefore, the antioxidant activity of hydrophilic and lipophilic compounds can be evaluated by ABTS Assay [17]. The preparation of ABTS solution using K2S2O8 as an oxidant to make ABTS radical cation [18]. The capability of electron or hydrogen donor resulted from the decolorization of ABTS solution from blue-green color to colourless solution. According to the samples, it showed that there was a significant difference between crude and purified liquid smoke. Crude liquid smoke derived from wood sawdust has a higher IC50 value than purified liquid smoke. This assured that crude liquid smoke exhibited effective antioxidant activity compared to the purified product. All the samples of liquid smoke scavenged ABTS radical in a concentration-dependent way (39.06 µg/mL - 625 µg/mL) (Table 1); the activity increased significantly as the result of concentration increased for each sample.

According to the classification of antioxidant activity, the antioxidant of crude liquid smoke is categorized as a strong antioxidant (less than 50 µg/mL) while purified liquid smoke is classified as a weak antioxidant (above 200 µg/mL) [19]. Furthermore, the antioxidant of crude liquid smoke was higher than ascorbic acid as a standard. Crude liquid smoke which has high antioxidant activity may be related to many active compounds specifically phenolic compounds [20] Several commercial liquid clouds of smoke are reported to have a value of IC50 ranging from 1.3 mg/mL (Liquid smoke S2) to 46.8 mg/mL [21].

The OH group that can be found in phenol compound have the capability to transfer H atom to chain carrier radicals which can reduce the amount of oxidation [22]. According to the total phenol content assay in this study, it was also found that the phenol content of crude liquid smoke was high with the value of TPC was 95.93 µgGAE ± 1.15 µgGAE, and it possesses significantly stronger ABTS scavenging potency. On the other hand, the antioxidant activity of purified liquid smoke had a weak scavenging activity, likewise, the phenolic content of purified liquid smoke was lower than crude liquid smoke. According to the other study, it was reported that the type of smoking process, smoke production conditions, application technique, temperature, and humidity strongly affect the concentrations of the phenolic compounds in smoked products [21]. Moreover, the purification of crude liquid smoke using zeolite and active carbon was also affected the number of phenolic compounds that can be attributable to toxic conditions. The chemical composition was also investigated using the GC-MS method. In this present study, the composition focused on their bioactivity and the quantitative determination of the phenolic compound. Crude liquid smoke had the higher composition of phenolic compound (12.77%) than purified liquid smoke (10.5%). There are two dominants of phenolic compounds in both crude and purified liquid smoke, Phenol (CAS) Izal and Phenol, 2-methoxy- (CAS) Guaiacol respectively. According to the bioactivity, it was reported that Phenol (CAS) Izal and Phenol, 2-methoxy- (CAS) Guaiacol have antioxidant activity [9] [10]. In another study conducted by Soldera *et al.*, 2008 [21]. Liquid smoke produces guaiacol as the main characteristic of phenolic compounds. Considering the composition of the phenolic compound and their antioxidant activity, it can be shown that the high concentration of the phenolic compounds resulted in high antioxidant activity. Thus, the presence of phenol could be the main factor affecting free radical scavenging activity. This aspect needs further investigation.

The results obviously demonstrate that theantioxidant potential of crude liquid smoke and purified liquid smoke in comparison with ascorbic acid showed significant differences. The antioxidant properties of crude liquid smoke derived from woodsawdust showed that crude had high antioxidantactivity with higher phenol content that potentialize as a food preservative.

AUTHORS' CONTRIBUTIONS

R. Suryani designed the experiments. Y. A. Purwestri leads and monitors the project collaboration. R. Daradwinta performed whole experiments, analyzed the data, and write the manuscript. S. Az-Zahra writes and edits the manuscript. S. A. Rahmah writes and edits the manuscript. W.A. Rizal performed a chemical composition analysis. T. H. Jatmiko performed phenolic analysis. W. Apriyana analyses the chemical composition.

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