Physicochemical Characteristics, Antioxidant and Antibacterial Activities of Liquid Smoke Derived from Mixed Sawdust and Cocoa Pod Husks Biomass

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Abstract

This study aimed to analyze the physicochemical characteristics, antioxidant and antibacterial activities of liquid smoke derived from mixed sawdust (MS) and cocoa pod husks (CPH) biomass. The liquid smoke formed by pyrolysis of MS and CPH biomass (*Theobroma cacao* L.) has the physical characteristics of brown color, transparrent, has floating solids, and has an acidic pH of 3.79 for MS and 5.43 for CPH. The results of the antibacterial assay of both liquid smokes by agar diffusion method against 3 pathogenic bacterial isolates; *Escherichia coli* FNCC 0091, *Staphylococcus aureus* FNCC 0047 and *Salmonella typhimurium* FNCC 0150 showed positive results with the formation of clear zones around the test discs. Antioxidant activity assay showed that liquid smoke derived from the biomass of MS had an IC50 value of 0.112 mg/mL, more potential than liquid smoke derived from the biomass of CPH, which was 2.060 mg/mL. Liquid smoke chemical composition analyzed by Gas Chromatography-Mass Spectrometry method showed that liquid smoke derived from MS biomass was dominated by acetic acid (64.64 %), phenols (12.08 %) and acetone (5.78 %), while the liquid smoke derived from CPH biomass was dominated by methylamine (37.26 %), acetic acid (24.61 %) and acetone (19.89 %). The acetic acid, phenols, methylamine and acetone content in both liquid smokes thought to play an important role on its antioxidant activities and inhibition of bacterial growth.

Keywords: Pyrolysis, Biomass, Waste, Antioxidant, Antibacterial

Introduction

Biomass is a renewable energy source that comes from animals, plants and microorganisms which has high volatile content but low carbon content. The high content of volatile compounds in biomass causes combustion to start at low temperatures. In general, biomass consists of cellulose (30 - 50 %), hemicellulose (15 - 40 %) and lignin (15 - 35 %). Cellulose and hemicellulose are relatively easy to metabolize, while lignin requires a high temperature in biochemical conversion with the remaining result in the form of ash [1-3].

The furniture industry produces solid waste in the form of MS which is classified as biomass. In the manufacture of furniture and home furnishings, the types of wood commonly used are teakwood and mahogany. Teak tree (*Tectona grandis*) is a type of tree that produces high-quality wood. Teak trees have straight stems, can grow large and reach a height of 30 - 40 m. Although it is hard and strong, teak wood is easy to cut and work with, so this kind of wood is widely used for making furniture, panels and carvings. Teak furniture is known to be very strong and durable, and the shape does not easily change due to weather changes in association with pleasant aesthetics [4]. Mahogany (*Toona sureni*) is a large tree with a height of 35 - 40 m and a diameter up to 125 cm. It has cylindrical straight stems and is buttless. The outer skin is blackish brown, grooved superficially like scales, the bark is gray and smooth while young, but turning dark brown, grooved and peeling off when matured. For the last 20 years, mahogany plants have been cultivated because of their high economic value. The quality of the wood is hard and excellent for furniture, carving items and handicrafts. So it is often dubbed the 2nd prima donna in the wood market. The MS as a waste of furniture and home furnishings manufacture is mostly sold without further processing and used as fuel for clay stoves.

CPH biomass is a waste product of the chocolate industry. Cocoa (*Theobroma cacao* L) pod has been described as a natural laminated material consisting of 3 distinctly different layers: Outer, middle and inner pericarp, respectively. The chocolate industry used the inner part of cocoa fruits in order to make food products while the pods are not yet utilized for specific purposes [5]. Even though cocoa is a potential source of minerals, fiber and antioxidants [6], only 10 % of the total cocoa fruit weight is used for its commercialization, while the remaining 90 % is discarded as waste or by-products [7]. Most of the CPH waste is only left to rot around the plantation area so the economic value obtained from the utilization is quite low.

Pyrolysis applies extremely high heat to biomass to smoldering (not burning) in order to produce 3 forms of substances, including solids in the form of charcoal, gases in the form of smoke and liquids in the form of tar and liquid smoke. Liquid smoke is obtained from the pyrolysis process with minimal oxygen, releasing a gas that appears as ordinary smoke. These gases are rapidly cooled in the condenser, thus liquefying the smoke [8]. The biomass used as a raw material to produce liquid smoke depends on its availability. MS and CPH are biomass widely available in Indonesia. MS as a by-product of furniture industry can be found in many cities in Indonesia, especially in Jepara (Jawa Tengah), Pasuruan (Jawa Timur) and Indramayu (Jawa Barat) [9]. It is approximated that the generated sawdust waste from Indonesia's sawmills can amount to 246,337 - 1,604,028 m/year, quite an abundant quantity [10]. For large and integrated industries, sawdust waste has been used to form charcoal briquettes and sold commercially. However, for the small industrial-scale sawmills, which are thousands in number and spread across rural areas, this waste has not been utilized optimally. CPH biomass is also abundantly available in Indonesia. According to statistical data from Indonesia in 2019, Indonesia's total cocoa production reached 774,200 tons, of which 769,000 tons were produced from large community plantations [11]. CPH as a waste material are estimated to reach 619,000 - 696,000 tons of the total cocoa produced.

Through the pyrolysis process, MS and CPH biomass are expected to have a higher economic value with the output in the form of liquid smoke. Liquid smoke can be used for various purposes, among them is to preserve or extend the shelf life of a product. The functional properties of liquid smoke are obtained from its organic compounds which include phenols, alcohols, carboxylic acids, terpenes, ketones and aldehydes [12]. Acid compounds contained in liquid smoke can inhibit the formation of spores and microbial growth in food products [13-16]. Meanwhile, the phenolic compounds of liquid smoke have antibacterial and antioxidant properties, and potential in exhibit microbial activity against various microorganisms such as bacteria [17,18]. This study aimed to determine the physicochemical characteristics, antioxidant and antibacterial activities of liquid smoke derived from MS and CPH biomass.

Materials and methods

Biomass preparation

MS used in this study was obtained from the furniture home industries in Gunungkidul, Daerah Istimewa Yogyakarta, Indonesia. CPH biomass obtained from Chocolate Home Industries unit in the Nglanggeran area, Gunungkidul, Daerah Istimewa Yogyakarta, Indonesia. The biomass of MS and CPH dried under the sun for 7 days. Moisture content affects the rate of heat rise in the pyrolysis process; higher water content reduces the heating process because most of the heat source will be used to evaporate the water content of raw material [19]. The moisture content of dried biomass was measured using a moisture analyzer (AND MX-50).

Lignocellulose content analysis

Lignocellulose content was analyzed using the Chesson method [20]. One g of dried sample of biomass (a) was added with 150 mL of distilled water and refluxed at 100 °C on a water bath for 1 h, then filtered by using filter paper no.1 (Whatman) to separate the residue and filtrate. Furthermore, the residue was washed using hot water and dried at 105 °C until a constant weight was obtained (b). The residual reflux (b) was added by 150 mL of 1 N H_2SO_4 and refluxed again in the water bath for 1 h at 100 °C. The reflux residue obtained was filtered, washed and dried until becomes dried residue (c). Then 10 mL of 72 % H_2SO_4 was added to the dried residue (c) and incubated at room temperature for 4 h. Furthermore, 150 mL of H_2SO_4 1 N was added and refluxed again in the water bath for 1 h. The residue was filtered, washed and dried (d). Then the residue (d) dusted and the remaining ashes are measured (e). Calculations to determine the lignocellulose composition of biomass are as follows cellulose = (c − d/a×100 %; lignin = (d − e)/a×100 %; water-soluble substance = (a − b)/a×100 %; hemicelluloses = (b − c)/a×100 %.

Biomass pyrolysis

Dried CPH were reduced in size to ± 1 - 5 cm using a grinder machine and its weight was adjusted to the capacity of the pyrolysis reactor [19]. Pyrolysis of MS biomass was conducted by this process parameters; an amount of 7.4 kg of MS biomass was placed in a pyrolysis reactor, then after the system is tightly closed, pyrolysis was carried out at a temperature of 530 °C for 8 h. Meanwhile, pyrolysis of CPH biomass was conducted in the same reactor with the weight of 7 kg sample, at the temperature of 525 °C for 8 h. The condensed product of pyrolysis in the form of liquid smoke was collected in a container and allowed to stand for 24 h to precipitate the tar.

Physicochemical properties analysis

The liquid smoke derived from MS and CPH biomass was analyzed to determine its degree of acidity using a pH meter (Mettler Toledo) and chemical composition using a GCMS. The GCMS (GC-2010/QP2010S, Shimadzu) was equipped with a DB-624 column (Agilent Technologies, Inc. 30 m×250 μm×1.40 μm). The carrier gas was helium. The injector temperature was 250 °C. The initial oven temperature of the column was 40 °C, which was maintained for 5 min, raised to 190 °C at 4 °C/min, and then maintained for 17.5 min at 190 °C. The ion source temperature and interface temperature were 240 °C. Ionization energy was 70 eV, and the mass range was m/z 28 AMU to 600 AMU. The total flow was 36 mL/min, column flow was 0.85 mL/min, and the linear velocity was 33.2 cm/s. The identification of chemicals in the samples was determined by comparing the spectra and retention time of the individual compounds with the authentic reference compounds stored in the mass spectral data library.

Color measurement

The color intensity of liquid smoke derived from MS and CPH biomass was measured using a Chromameter color reader (Konica Minolta CR-20, Japan) with a measuring area of 8 mm in diameter. The values of L^*, a^* and b^* were obtained by 3 repetitions for each test sample.

Fourier-transform infrared spectra analysis of brown pigment

The liquid smoke derived from MS and CPH biomass is dropped for the spectra analysis using FTIR (Bruker vertex 80) with the range of 4.000 - 400 cm^{-1} [21,22].

Antioxidant assay

The antioxidant activity of liquid smoke was determined by ABTS 2,2′-Azino-bis (3-ethyl benzthiazoline-6-sulfonic acid) method [23]. 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 h at room temperature and protected from light exposure. Tests were carried out using 96 well plates (Nunc). Each sample of liquid smoke was made of stock solution with a concentration of 1,000 mg/mL. Then the stock solution was diluted to a concentration of 500, 250, 125, 62.5, 31.25, 15,625 and 7,8125 mg/mL. In each well 15 μL of sample was put in and then added by 285 μL of 7.4 mM ABTS solution. At each sample concentration, 3 replicates were performed. Amount 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 the dark place. Elisa reader (Multiskan™ FC Microplate Photometer) at a wavelength of 734 nm was used to measure absorbance. Ascorbic acid (Merck) was used as a positive control with an IC 50 value of 0.06 mg/mL.

Antibacterial assay

Antibacterial assay was carried out by referring to the method recommended by CLSI [24]. Pure culture of bacterial isolates *Escherichia coli* FNCC 0091, *Staphylococcus aureus* FNCC 0047 and *Salmonella typhimurium* FNCC 0150 each scratched separately on a petri dish for 24 h to form a single colony. The single colony formed was taken with a sterile ose and put in a test tube containing of 0.85 % sterile NaCl and stirred until homogeneous. The mixture was then equilibrated to the McFarland standard of 0.5 and used as inoculum. Sterile cotton buds are used to smear the inoculum evenly on the surface of Mueller Hinton Agar medium (Himedia). Sterile blank discs (Oxoid) were placed with sterile tweezers on a medium that had been scratched with bacterial inoculum and 10 μL of each liquid smoke sample was dropped. Ampicillin 10 mcg (Oxoid) was used as a positive control. Incubation was carried out for 18 - 24 h at 37 °C. Each test was replicated 2 times. The diameter of the clear zone formed around the disc was measured using a digital caliper (Krisbow).

Minimum inhibitory concentration (MIC) assay

Measurement of the MIC was carried out by referring to the method [25] with some modification. Pure culture of bacterial isolates *Escherichia coli* FNCC 0091, *Staphylococcus aureus* FNCC 0047 and *Salmonella typhimurium* FNCC 0150 was used as test bacteria. The test was carried out in a 96-well plate (Nunc) with serial dilutions of liquid smoke in various concentrations ranging from 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39 and 0 % (negative control) in sterilized Nutrient Broth (Merck) medium for *Escherichia coli* FNCC 0091 and *Staphylococcus aureus* FNCC 0047, and in sterilized Tryptic Soy Broth (Merck) medium for *Salmonella typhimurium* FNCC 0150. The test bacterial culture used as inoculum was prepared by adding one loop of bacteria colony that have been rejuvenated for 24 h into sterilized Phosphate Buffer Saline (Oxoid) and equalized for turbidity according to the McFarland standard of 0.5 (approximate cells density 1.5×10^8 cells/mL). Each well was filled with a volume of 150 µL of diluted sample followed by addition of 50 µL of test inoculum. Ampicillin 0.1 % (Sigma) was used as a positive control. Incubation was carried out for 18 - 20 h at 37 °C. The MIC is the lowest concentration of liquid smoke which prevents visible growth of a test bacteria.

Results and discussion

Biomass contents

MS and CPH used as raw materials for producing liquid smoke have differences in moisture and lignocellulose content (lignin, cellulose and hemicellulose). The lignocellulose content of the 2 biomasses were presented in **Table 1**.

Table 1 Lignocellulose content of MS and CPH biomass as pyrolysis materials.

Components	Percentage (%)	
	MS	**CPH**
lignin	25.59 ± 1.60	18.22 ± 0.98
cellulose	39.97 ± 1.33	19.53 ± 0.73
hemicelluloses	17.54 ± 2.59	20.22 ± 2.31
water soluble substance	16.90 ± 0.24	42.19 ± 2.35

CPH has higher lignin and hemicelluloses contents than MS but lower in cellulose content. High-temperature pyrolysis can cause degradation of cellulose, hemicelluloses and lignin which occurs in 4 stages, starting with water evaporation, followed by hemicelluloses decomposition, cellulose decomposition and lignin decomposition. Decomposition of hemicelluloses and cellulose occurs at temperatures between 180 to 350 °C [8], resulting in carboxylic acids and carbonyl compounds, while lignin decomposition occurs at temperature between 300 and 500 °C and generate phenols [3,26].

Liquid smoke characteristics

The characteristics of liquid smoke derived from MS and CPH biomass are presented in **Table 2**.

Table 2 Physicochemical characteristic of liquid smoke derived from MS and CPH biomass.

Characteristics	MS			CPH		
color	L^*	a^*	b^*	L^*	a^*	b^*
	51.77	6.67	39	49.77	6.94	35.24
transparency	transparent			transparent		
floating substance	exists			exists		
pH	3.79			5.43		

The color of liquid smoke originated from pyrolysis process of MS and CPH biomass both are dark brown (**Table 2**). The brown color of liquid smoke influenced by the carbonyl compounds, the higher carbonyl contents the higher the browning potential [27]. Each liquid smoke has an acidic pH, with liquid

smoke derived from MS having lower pH value, which indicates more organic acid content. Color measurement resulted from the liquid smoke derived from MS has a higher L (Lightness) and b value than liquid smoke derived from CPH biomass. L value represents darkness from black (0) to white (100), the a value represents color ranging from red (+) to green (–), and the b value represents yellow (+) to blue (–). To determine the total color difference (ΔE^*) between the 3 coordinates, the following formula is used:

$$\Delta E^*_{ab} = \sqrt{\Delta L *^2 + \Delta a *^2 + \Delta b *^2} \tag{1}$$

$$\Delta E^*_{ab} = \sqrt{2 *^2 + (-0,27 *)^2 + 3,76 *^2} \tag{2}$$

$$\Delta E^*_{ab} = 4,27 \tag{3}$$

The total color difference (ΔE^*) value of liquid smoke derived from MS and CPH biomass is 4.27, which is categorized as 'clear difference in color is noticed [28].
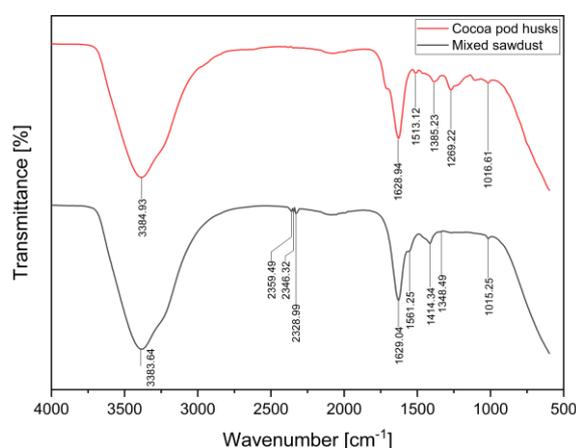
Figure 1 FTIR spectra of liquid smoke derived from MS and CPH biomass.

Based on **Figure 2**, a broad band in the range of 3383.64 and 3384.3 cm^{-1} of both liquid smokes indicates the hydroxyl group. There is no bond identified in the triple bond region (2,000 - 2,500 cm^{-1}), informing no C≡C bond in the sample [22]. Liquid smoke derived from MS's hydroxyl compound followed by the strong signal at frequencies 1,629.04 and 1,561.25 cm^{-1}, which is responding double bonds or aromatic compounds, and at frequency 1,414.34 cm^{-1} possibly describing phenol or tertiary alcohol functional group. Liquid smoke derived from CPH biomass's hydroxyl compound followed by the spectra at frequencies 1,628.94 and 1,513.2 cm^{-1} which is possibly describing double bonds or aromatic compounds, spectra at frequency 1385.23 cm^{-1} possibly describing Methyl-functional group, while frequency 1,269.22 cm^{-1} possibly describing phenol with C-O stretch, and frequency 1,016.61 cm^{-1} possibly describing primary alcohol, with C-O stretch or aromatic C-H in-plane bend. Identification of main chemical composition of both liquid smokes based on GCMS analysis are shown in **Table 3**.

Table 3 Chemical composition of liquid smoke derived from MS and CPH biomass.

Chemical composition	Percentage (%)	
	MS	**CPH**
Acetic acid	64.64	24.34
Propanoic acid	5.77	1.54
Butyric Acid	1.3	-
Butanoic acid	-	0.67
Isobutyric Acid	0.24	-

Chemical composition	Percentage (%)	
	MS	CPH
Methylamine	-	37.26
1-hydroxy-2-Propanone	5.01	3.06
Acetone	0.77	16.38
1-Hydroxy-2-butanone	1.76	0.55
Phenol	4.71	2.91
2-methoxy-phenol	3.65	1.54
3-methyl-phenol	1.92	0.98
2,3-dimethyl-phenol	0.26	-
2-methoxy-4-methyl-phenol	0.77	-
4-ethyl-2-methoxy-phenol	0.40	-
2,6-dimethoxy-phenol	0.37	0.87
2-hydroxytetrahydrofuran	0.31	-
1,6-Heptadien-4-ol	0.24	-
Butyrolactone	1.28	2.33
Furfuryl Alcohol	-	2.48
2-Cyclopenten-1-one	1.54	0.52
3-Methyl-2-Cyclopenten-1-One	1.54	0.97
others	3.52	3.60

Based on **Table 3**, the acetic acid content of liquid smoke derived from MS was higher than liquid smoke derived from CPH biomass. This corresponds with the acidity of liquid smoke, were liquid smoke derived from MS has more acidic pH value (pH 3.79) than liquid smoke derived from CPH biomass (pH 5.43). This phenomenon was influenced by the cellulose content of each biomass. Through pyrolysis, cellulose will undergo hydrolysis which produces glucose, and the subsequent reaction produces water, acetic acid and a small amount of phenol [3,27]. Since cellulose content in MS biomass was higher than CPH, thus the acetic acid content in MS liquid smoke was also higher than CPH.

Phenols content of liquid smoke derived from MS were higher (12.08 %) and varied than phenols content of liquid smoke from CPH biomass (6.3 %). This phenomenon corresponded with the higher lignin content in MS biomass than CPH because pyrolysis process of lignin produced phenol compounds and their derivatives [3]. In other side, liquid smoke derived from CPH containing methylamine (37.26 %) and acetone (16.38 %), while liquid smoke derived from MS did not contained any methylamine and having small amount of acetone (0.77 %). Compounds like phenols, carbonyls and organic acids are the results of wood pyrolysis which are responsible for the flavor, color and antimicrobial properties of liquid smoke [13].

Antioxidant activities
Results of antioxidant assay of liquid smoke derived from MS and CPH biomass were shown in **Table 4**.

Table 4 Antioxidant activity of liquid smoke derived from MS and CPH biomass.

Sample	Linear regression equation	IC50 (mg/mL)
MS	$y = 0.0375x + 45.767$ $R^2 = 0.9454$	0.112
CPH	$y = 0.0054x + 38.873$ $R^2 = 0.9471$	2.060

Based on the results presented in **Table 4**, the IC50 of liquid smoke derived from MS and CPH were 0.112 and 2,060 mg/mL, respectively. Liquid smoke derived from MS considered as strong antioxidant activity, while liquid smoke derived from MS considered as weak activity. The liquid smoke derived from MS has higher antioxidant activity than CPH due to higher phenolic compounds content in MS smoke liquid. Phenolic compounds are an important group which have antibacterial and antioxidant properties [29-31]. This result shows possible correlation between the phenolic contents of liquid smokes and their antioxidant activities. Phenolic compounds found in both liquid smokes are Phenol, 2-methoxy-phenol, 3-methyl-phenol, 2,3-dimethyl-phenol, 2-methoxy-4-methyl-phenol, 4-ethyl-2-methoxy-phenol and 2,6-dimethoxy-phenol.
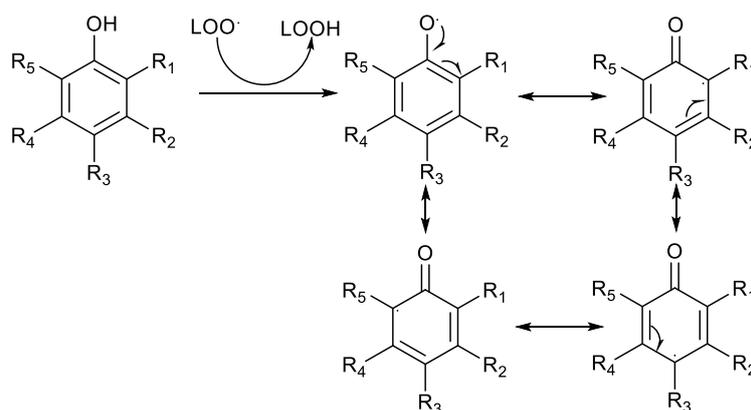
Figure 2 The reaction mechanism of phenolic compounds with free radicals and their stabilization.

Table 5 The Purpose Mechanism of Phenolic Compounds with Free Radicals in Liquid Smokes.

Phenolic compounds	R_1	R_2	R_3	R_4	R_5
2-methoxy-phenol	OCH_3	H	H	H	H
3-methyl-phenol	H	CH_3	H	H	H
2,3-dimethyl-phenol	CH_3	CH_3	H	H	H
2-methoxy-4-methyl-phenol	OCH_3	H	CH_3	H	H
4-ethyl-2-methoxy-phenol	OCH_3	H	CH_2CH_3	H	H
2,6-dimethoxy-phenol	OCH_3	H	H	H	OCH_3

An antioxidant is any substance that delays or inhibits the oxidation of the substrate (**Table 5**). Phenolic compounds in liquid smoke such as 2-methoxy-phenol, 3-methyl-phenol, 2,3-dimethyl-phenol, 2-methoxy-4-methyl-phenol, 4-ethyl-2-methoxy-phenol and 2,6-dimethoxy-phenol have an antioxidant activity through hydrogen atom transfer (HAT) or single-electron transfer mechanism [32]. The structure of phenolic compounds, particularly the benzene ring and the amount and position of hydroxyl group (OH) groups, determines their capacity or potency to act as antioxidants. Following an interaction with free radicals, the benzene ring is in charge of stabilizing antioxidant molecules. However, the hydroxyl group serves as an antioxidant by producing phenolic acid-free radicals [33,34]. The resonance effects of the aromatic ring stabilize the radical (**Figure 3**).

Antibacterial activities

Antibacterial activity of crude liquid smoke was identified using various pathogenic bacteria that consisted of *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhimurium*. E. *coli* and S. *typhimurium* are Gram-negative bacteria. E. c*oli* naturally live-in humans and other animal's intestine. Most E. *coli* are harmless, but some strain can cause serious food poisoning and severe illness. Salmonella is a food borne bacterium. Salmonella contamination especially prevalent in eggs and raw poultry, but the bacteria also can contaminate various foods including raw vegetables, fruits, meat, beef, chicken, pork and even processed foods. *Staphylococcus aureus* is a gram-positive, sphere-shaped (coccal) bacterium, present

in the nose (usually temporarily) and on the skin. The bacteria can cause skin infections, heart valve infections, pneumonia and bone infections.

The results of antibacterial activity of liquid smoke derived from MS and CPH biomass were shown in **Table 6**.

Table 6 Antibacterial activities of liquid smoke derived from MS and CPH biomass.

Sample	Average of clear zone diameter (mm)		
	Escherichia coli **FNCC 0091**	*Staphylococcus aureus* **FNCC 0047**	*Salmonella typhimurium* **FNCC 0150**
MS	12.35 ± 0.405^b	8.73 ± 0.890^b	13.36 ± 0.260^b
CPH	10.95 ± 0.485^b	8.87 ± 0.625^b	12.81 ± 0.340^b
Ampicillin 10 mcg	17.80 ± 0.470^a	12.31 ± 0.03^a	16.53 ± 0.020^a

Based on the results presented in **Table 3**, liquid smoke derived from MS and CPH biomass shows inhibitory activity toward bacteria *Escherichia coli* FNCC 0091, *Staphylococcus aureus* FNCC 0047 and *Salmonella typhimurium* FNCC 0150 with a clear circle formed around the test disc. Liquid smoke derived from MS has greater inhibition diameters than liquid smoke derived from CPH biomass. The strength of antibacterial activity is categorized as follows: Inhibition area > 20 mm is very strong, inhibition area of 10 - 20 mm is strong, inhibition area of 5 - 10 is moderate, and inhibition area < 5 mm is in the weak category [35]. Thus both liquid smokes have strong category antibacterial activity against *Escherichia coli* FNCC 0091 and *Salmonella typhimurium* FNCC 0150, and moderate category against *Staphylococcus aureus* FNCC 0047.

Table 7 MIC value of liquid smoke derived from MS and CPH biomass.

Sample	Minimum inhibitory concentration (MIC) value		
	Escherichia coli **FNCC 0091**	*Staphylococcus aureus* **FNCC 0047**	*Salmonella typhimurium* **FNCC 0150**
MS	0.39 %	0.78 %	1.56 %
CPH	3.125 %	6.25 %	6.25 %

MIC assay shows that liquid smoke derived from MS biomass has lower MIC values than liquid smoke derived from CPH (**Table 7**) in all tests against 3 pathogenic bacteria, namely *Escherichia coli* FNCC 0091, *Staphylococcus aureus* FNCC 0047 and *Salmonella typhimurium* FNCC 0150. This result confirms that liquid smoke originated from MS has more antibacterial potential than liquid smoke originated from CPH biomass. MIC value of liquid smoke derived from MS against *Escherichia coli* FNCC 0091 is 0.39 %, while the liquid smoke derived from CPH has MIC value of 3.125 % against similar bacteria. MIC value of liquid smoke derived from MS against *Staphylococcus aureus* FNCC 0047 is 0.78 %, while the MIC value of the liquid smoke derived from CPH is 6.25 %. MIC value of liquid smoke derived from MS against *Salmonella typhimurium* FNCC 0150 was 1.56 %, while liquid smoke from CPH could inhibit the growth of *Salmonella typhimurium* FNCC 0150 at a higher concentration of 6.25 %.

An MIC value refers to the minimum concentration of an antimicrobial substance that inhibits the growth of microorganisms [36]. In this study, the MIC is the lowest concentration of liquid smoke which prevents visible growth of a test bacteria. Previous study shown growth inhibition of liquid smoke against S. *aureus* and S. *choleraesuis* with 10 % liquid smoke concentration, and for the E. coli bacteria there have not been cellular growth in concentrations of up to 7.5 % of smoke [17]. MICs investigation of 8 commercial liquid smoke samples from different wood sources against *Salmonella enteritidis*, *Staphylococcus aureus* and *Escherichia coli* was resulted the ranged from 0.5 to 6.0 % for E. *coli*, 0.5 to 8.0 % for Salmonella and 0.38 to 6 % for S. *aureus* [37].

Liquid smoke compounds which have potential ability to inhibit growth of spoilage and pathogenic microorganisms are phenolic compounds and organic acids [8,13]. Phenolic compounds such as 2,6-dimethoxy-4-methylphenol, 2.6 dimethoxy phenol and 2.6-dimethoxy-4-ethylphenol have high bactericidal activity. Phenols inhibit the growth of bacteria by hyper acidification at the plasma membrane interphase [38] because of dissociation of phenolic acids. The gram-positive bacterium lacks an outer membrane,

which would allow the phenolic acids to diffuse through the cell wall and intracellular acidification, as well as irreversible alterations in the sodium-potassium ATPase pump, hence leading to cell death. The outer membrane of gram-negative would act as a barrier to hyper acidification. Among the various organic acids such as formic, acetic, propionic, butyric and isobutyric acids, acetic acid has the stronger bactericidal activity. Acetic acid inhibits bacterial growth through the mechanism of disruption of bacterial cell membranes, and inhibits the synthesis of enzymes and various macromolecules in cells [39]. Organic acids are anionic surfactants that act as disinfectants that disrupt membrane stability. The general mechanism of inhibition of microbial growth by organic acids is through acidification of the cell cytoplasm caused by the release of excess protons after acid dissociation [40]. Liquid smoke derived from MS has more acidic pH and greater number of phenols compounds than liquid smoke derived from CPH biomass. These results possible has closely related with their antibacterial activity toward pathogenic bacteria; liquid smoke derived from MS had more potential than liquid smoke derived from CPH biomass.

Conclusions

Liquid smoke derived from MS and CPH biomass has characteristics including dark brown color, transparent, have floating materials, and acidic pH value. Chemical composition analysis of liquid smoke by GCMS method resulted that liquid smoke derived from MS had acetic acid (64.64 %) and phenols contents (12.08 %), higher than liquid smoke derived from CPH with acetic acid (24.34 %) and phenols (6.3 %). Antioxidant activity determined by ABTS method resulted the IC50 values, with the IC50 of liquid smoke derived from MS had 0.112 mg/mL, and liquid smoke derived from CPH had IC50 2,060 mg/mL, respectively. Liquid smoke derived from MS considered as strong antioxidant activity, while liquid smoke derived from MS considered as weak activity. Antibacterial assay followed by MIC assay against bacteria *Escherichia coli* FNCC 0091, *Staphylococcus aureus* FNCC 0047 and *Salmonella typhimurium* FNCC 0150 showed that liquid smoke derived from MS had more antibacterial potential than liquid smoke derived from CPH biomass, due to more acidic pH and greater number of phenols compounds.

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