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Physical and Phytochemical Properties of Coconut Shell Liquid Smoke: Antibacterial and Antioxidant Alternative

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Abstract. Coconut shell liquid smoke originates from smoke condensation by the decomposition of organic compounds in pyrolysis. The pyrolysis yield of cellulose, hemicellulose, and lignin compounds produce organic acid, phenols, and carbonyls, playing the preservation and antioxidant roles. This research aims to determine the physical properties and chemical compounds contained in coconut shell liquid smoke, antioxidant activity (IC50), and antibacterial activity. The organoleptic observation was performed to identify the physical properties. Meanwhile, phytochemical screening and GCMS were utilized to determine the chemical content. The DPPH method was adopted to ascertain the IC50, with quercetin as the standard. The antibacterial activity was evaluated using *E. coli* and the paper disc method, at varying concentration from 40 to 200 mg mL^{-1}. The positive control utilized 10 mg mL^{-1} amoxicillin, while the negative control employed sterile distilled water. The physical observations unveiled that it was a yellow liquid with a distinctive smell. The phytochemical analysis revealed positive saponins and flavonoids. The GCMS analysis disclosed the dominance of phenolic compounds. The antioxidant activity exhibited an IC50 of 87.40 mg mL^{-1}, categorized as a potent antioxidant. In comparison, the quercetin generated an IC50 of 12.7 mg mL^{-1}, falling into a very strong antioxidant.

1 Introduction

Indonesia is an archipelagic country with extraordinary natural wealth in the diversity of flora and fauna, including the coconut tree (*Cocos nucifera* L.). Coconuts offer various benefits, from fruit flesh, coir, shell, leaves, and stems [1]. The enormous demand for coconuts in the community has escalated the amount of coconut waste, such as coconut shells [2,3]. Hence, a substantial amount of coconut shell waste can be transformed into other materials for more valuable applications. This coconut shell waste can be utilized to produce liquid smoke [4,5], which can be obtained through steam condensation from pyrolysis [6].

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Liquid smoke derived from coconut shells is the result of smoke condensation—the decomposition of organic compounds in pyrolysis [6] Liquid smoke contains antibacterial, antioxidant, and antifungal compounds [7,8]. Accordingly, it has a wide range of applications, covering the food industry as a preservative, health industry, plant fertilizer, bioinsecticide, pesticide, disinfectant, herbicide, and so on. The antioxidant role of liquid smoke is demonstrated by phenol and acetic acid compounds, acting as hydrogen donors against free radicals and inhibiting chain reactions [9]. Acetic acid in liquid smoke also plays a vital role as an antibacterial [10,11].

The utilization of liquid smoke is distinguished by its quality [12,13]. Grade 1 liquid smoke is characterized by a pale-yellow color. This liquid smoke is suitable as a preservative in ready-to-eat foods such as wet noodles, meatballs, and tofu. Grade 2 liquid smoke has a brownish-yellow color. This liquid smoke preserves raw food ingredients such as meat, chicken, and fish [14]. Grade 3 liquid smoke is distinguished by its thick brown appearance and sharp smell. This liquid smoke is utilized in processing deodorizing rubber and wood preservatives.

Phenol and acetic acid act as antioxidants. These two act as hydrogen donors against free radicals and inhibit the reaction [9]. Antioxidants are compounds that can slow down the oxidation of food [15] Liquid smoke can be antibacterial. Phenol and acetic acid, contained in coconut shell liquid smoke, act as the antibacterial agent. Phenol will damage the cytoplasmic membrane, causing membrane leakage, leading to interfering bacterial growth, and even causing death [16–18]. Acetic acid in liquid smoke also plays a crucial role, leading to its utilization as an antibacterial [8]. Therefore, acetic acid can lead to the destabilization of various functions and structures of cell components [19].

Escherichia is one of the main Gram-negative bacterial species that lead to gastrointestinal infections [20]. These bacteria are found in the human intestine, which removes waste substances in the digestive tract and can infect the intestines, causing diarrhea [21]. An antibacterial agent can inhibit bacterial growth and kill pathogenic bacteria [22].

This study aims to determine the physical properties and chemical compounds of coconut shell liquid smoke, antioxidant activity (IC50), and antibacterial activity of coconut shell liquid smoke against *E. coli* bacteria.

2 Research method

2.1 Material

Coconut shell liquid smoke was obtained from liquid smoke treatment in Klaten Regency. Meanwhile, *E. coli* bacteria were collected from the Agrotechnology Laboratory of Universitas Muhammadiyah Yogyakarta. The bacterial media of MHA, HCl, H_2SO_4, $FeCl_3$, phenolphthalein, NaOH, DPPH, methanol, and quercetin were purchased from Merck, Germany. Amoxicillin liquid injection was attained from Kimia Farma Indonesia.

2.2 Experiments

2.2.1 Physical properties evaluation

Organoleptic observations of liquid smoke were conducted using the five senses by pouring it into a test tube. Liquid smoke pH measurements were carried out using pH paper, where the universal pH paper was dipped in 10 mL of liquid acid and placed in a reaction tube. The acid percentage was calculated based on the titration method by dissolving 10 mL of liquid smoke with 100 mL of distilled water and then homogenizing it. Subsequently, two to three drops of phenolphthalein indicator were utilized to determine the endpoint of the titration. The 0.1 N NaOH solution was employed as a titrant. The endpoint of the titration was marked by the color of the sample changing to purplish-red and stable [23–25]. Total acid was expressed as percent acetic acid (equation 1). *V* refers to the volume of NaOH, *N* indicates the concentration of NaOH, *MW* signifies the molecular weight of molecular acid, and *SW* denotes the sample weight (grams).

$$\frac{V \times N \times MW}{SW \times 1000} \times 100\% \tag{1}$$

2.2.2 Phytochemical evaluation

The alkaloid content test was carried out by adding 2 mL of coconut shell liquid smoke and two drops of Dragendorff's reagent. If a brown or orange precipitate forms at the bottom of the test tube, the sample contains alkaloids.

The saponin test was performed by adding 2 mL of liquid coconut shell smoke and 10 mL of hot water, cooling it, and then shaking it vertically vigorously for 10 seconds. Subsequently, one drop of 2 N HCl was added. If stable foam forms, the sample contains saponin.

The flavonoid test was run by adding 2 mL of coconut shell liquid smoke and 10% NaOH solution and then observing the color change. If the color changes to reddish yellow, it is positive for flavonoids [26].

2.2.3 Identification of phytochemical compounds with GCMS

The analysis was performed with a gas chromatography-mass spectrophotometer (GC-MS) model Shimadzu QP-5000, with an ionizing electron impact (EI) type of 70 eV and a 30 m capillary column containing UHP Helium (He). The injector temperature was 290 °C, and the separation temperature was programmed from 50 to 250 °C in increments of 10 °C per minute. As a carrier gas, helium gas was employed with a pressure of 20 kg per cm^2 and a flow velocity of 0.2 mL min^{-1}. The liquid smoke was dissolved in ether; a separation was made between the ether-soluble and polar phases. Subsequently, 50 µL of ether phase was taken and injected into GC-MS.

2.3 Antioxidant activity test

The antioxidant activity of coconut shell liquid smoke was examined using the DPPH method [7]. The first antioxidant activity test was preparing a 100 mg mL^{-1} DPPH solution by weighing 25 mg of DPPH powder and dissolving it in analytical-grade methanol up to 100 mL. The second step was the determination of the maximum wavelength using 1 mL of methanol and 4 mL of DPPH solution placed into a test tube and mixed. The cuvette was filled with 3 mL of prepared methanol as a blank. Then, UV-VIS spectrophotometry was utilized to measure wavelengths at 200-800 nm.

Furthermore, the liquid smoke stock solution at a concentration of 1,000 mg mL^{-1} was prepared. Series concentrations were made into 40, 80, 120, 160, and 200 mg mL^{-1}, which were further analyzed. The quercetin solution as a comparison was made in concentration variations of 1, 3, 5, 7, and 9 mg mL^{-1}.

Furthermore, the antioxidant activity against each sample was determined, with quercetin serving as the standard. To assess the antioxidant activity of each test solution, 2 mL of DPPH solution and 6 mL of analytical-grade methanol were mixed and then incubated in a dark room for 30 minutes, and the absorbance was at the optimum wavelength. The IC50 value was obtained by plotting percent inhibition and concentration.

2.4 Antibacterial activity test

The antibacterial activity of liquid smoke against *E. coli* bacteria was observed using disc diffusion. All tools and materials (except liquid smoke) were sterilized using an autoclave for 30 minutes at a temperature of 121°C. The media was prepared by dissolving 20 g of MHA media into 100 mL distilled water, homogenizing it, and heating it to boiling and sterilization. Bacterial inoculation was performed by placing a pure culture colony of *E. coli* bacteria using sterile inoculating loops into an MHA medium and then incubating it at 37 °C for 24 hours [27]. Antibacterial testing was carried out by varying the smoke liquid concentration from 40, 80, 120, and 160 to 200 mg mL^{-1}. The distilled water was utilized as the negative control, and 10 mg mL^{-1} amoxicillin was employed as the positive control. All tests were conducted for three replications. The inhibitory zone was calculated based on equation formula (2), where *Dv* denotes the diameter of the vertical inhibition zone, *Dh* represents the diameter of the horizontal inhibitory zone, and *Dc* refers to the diameter of the resistance zone of the paper disc.

$$\frac{(Dv-Dc)+(Dh-Dc)}{2} \tag{2}$$

3 Results and discussion

3.1 Analysis of Physical and Chemical Properties of Liquid Smoke

3.1.1 Observation of physical properties

Observations of coconut shell liquid smoke uncovered that it was yellow in color, had a distinctive odor, and had a liquid form. The coconut shell liquid smoke was proven to have a characteristic odor, color, and ability to inhibit growth in fungi, as well as a specific taste. Fig. 1 exhibits the physical coconut shell liquid smoke.

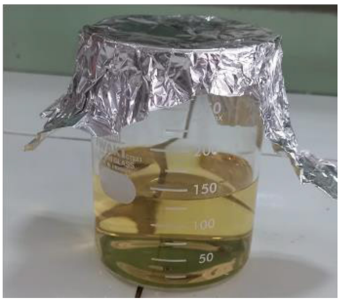


Fig. 1 Physical coconut shell liquid smoke

3.1.2 pH test

The pH value indicates the degree of decomposition of wood components in producing organic acid in liquid smoke. The test acquired a low pH value of 2. The results of this study meet Japanese liquid smoke quality standards, falling in the range of 1.55-3.70. This value implies that the liquid smoke produced from the coconut shell is acidic [28].

3.1.3 Acid Content

Two repetitions were carried out, generating an acid level of 4.82%. The acid contained in liquid smoke also comes from cellulose pyrolysis [29,30].

3.2 Phytochemical Screening

The testing of alkaloid compounds with reagents demonstrated changes. Specifically, the absence of orange deposits indicated that coconut shell liquid smoke contained alkaloid compounds. The testing of saponin compounds disclosed positive results, characterized by stable foam formation upon the addition of hot water and two drops of concentrated HCl, followed by shaking, confirming the presence of saponin compounds. The flavonoid compound test unveiled positive coconut shell liquid smoke. Table 1 exhibits phytochemical screening.

Table 1. Phytochemical screening of coconut shell liquid smoke

Screening	Result	Remark
Alkaloid		NEGATIVE No yellowish-white precipitation
Saponin		POSITIVE There was 1 cm high foam, and it lasted for 10 minutes after the solution was shaken.
Flavonoid		POSITIVE Formation of a red solution

3.3 Analysis of Liquid Smoke Chemical Components Using GCMS

Based on the library from the Mass Spectra data system, 44 compounds constituting liquid smoke were identified. However, only three peaks displayed sufficient abundance: peak number 4 with a retention time of 5.75 minutes and an area percentage of 60.77, peak number

9 with a retention time of 8.39 minutes and a percent area of 9.23, and peak number 15 with a retention time of 15.72 and an area percentage of 9.30. The peak with a frequency of 5.75 was phenol or phosphonic acid (p-hydroxyphenyl)-, or 2-vinylfuran. Meanwhile, at a retention time of 8.39 minutes, the compounds comprised formic acid, 2,6-dimethoxyphenyl ester, or phenol 2,6-dimethoxy- or 2,4-dimethoxyphenol. Additionally, at a retention time of 15.72, phenol, 2-methoxy- or mequinol, or 2-cyclopenten-1-one, 3,5,5-trimethyl- were identified. Fig. 2 displays the GCMS chromatogram.

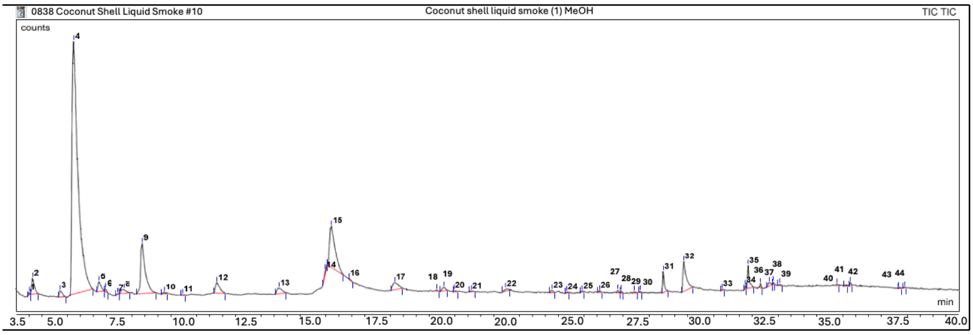


Fig. 2. GCMS chromatogram of coconut shell liquid smoke

The highest compound contained in coconut shell liquid smoke was phenol or phosphonic acid (p-hydroxyphenyl)-, or 2-vinylfuran, detected at a concentration of 60.77%, with a retention time of 5.75. Phenol is also referred to as carbolic acid, benzenol, and hydroxybenzene, as indicated by the Pubcem website and the National Institute of Standards and Technology (NIST) webbook. It is an antioxidant compound identified in liquid smoke, extending the shelf life of smoked products. Additionally, phenol provides a distinctive taste and color to processed products. Phosphonic acid compound (p-hydroxyphenyl) is also known as 4-hydroxyphenylphosphonic acid, with a molecular formula of $C_6H_7O_4P$ and a molecular weight of 174 g mol^{-1}.

It is an organic compound employed in the analysis of carbamic acid. The 2-vinylfuran compound, with a molecular formula of C_6H_6O and a molecular weight of 94 g mol^{-1}, was an organic compound with potential antimicrobial activity.

At a retention time of 15.72 minutes, the formic acid compound, 2,6-dimethoxyphenyl ester, with a molecular weight of 182 g mol^{-1}, was detected; it is an ester of formic acid (HCOOH), with 2,6-dimethoxyphenol ($C_8H_{10}O_3$). It was one of the components produced during combustion. Phenol or 2,6-dimethoxy-, known by other names such as pyrogallol 1,3-dimethyl ether, syringol, and 1,3-dimethoxy-2-hydroxybenzene, had a molecular weight of 154 g mol^{-1}. This compound can act as a flavor enhancer. Additionally, 2,4-dimethoxyphenol ($C_8H_{10}O_3$), known by other names such as 2,4-DMP, 2,4-dimethoxyhydroquinone, and 2,4-dimethoxybenzene-1,4-diol, had a molecular weight of 154 g mol^{-1}.

At a retention time of 8.39 minutes, phenol, 2-methoxy-, mequinol, and 2-cyclopenten-1-one, 3,5,5-trimethyl- were detected. Phenol, 2-methoxy-, with a molecular formula of $C_7H_8O_2$, is also called guaiacol. This compound had a molecular weight of 124 g mol^{-1}, contributing to a distinctive aroma, color, and taste to food products preserved using coconut shell liquid smoke [31]. Mequinol, with a molecular formula of $C_7H_8O_2$ and a molecular weight of 124 g mol^{-1}, is also known as 4-methoxyphenol. This compound has been frequently utilized to treat pigment disorders, such as melasma and post-inflammatory hyperpigmentation (PIH). Moreover, 2-Cyclopenten-1-one, 3,5,5-trimethyl-, had a molecular weight of 124 g mol^{-1}, which is a cyclopentenone derivative with various potential applications in the chemical and perfume industries.

3.4 Antioxidant Activity Test using the DPPH Method

The antioxidant determination was performed at a wavelength of 515.5 nm, which is the wavelength of DPPH in the analytical grade methanol, as recommended by previous researchers [32]. The antioxidant activity test was conducted using coconut shell liquid smoke, with concentration variations of 40, 80, 120, 160, and 200 mg mL^{-1}. Distilled water was employed as the negative control, while quercetin was utilized as the positive control. The data experiment disclosed that the antioxidant activity (IC50 value) of coconut shell liquid smoke was 87.40 mg mL^{-1}, illustrating potent antioxidant activity. It can be seen from the high content of antioxidant compounds, such as saponins and flavonoids. In addition, antioxidants also play a role in warding off free radicals. In this study, quercetin was deployed as a comparison. Quercetin is one of the flavanonol compounds, a flavonoid derivative with a 3-hydroxyflavone skeleton. Testing of quercetin solutions was carried out for three replications, generating an IC50 value of 12.7 mg mL^{-1}, categorized as a robust antioxidant. Table 2 summarizes the antioxidant data.

Table 2. Antioxidant Activity of Coconut Shell Liquid Smoke

Sample	Replication	IC50 (mg mL^{-1})	Average IC50 (mg mL^{-1})	SD	Average IC50 ± SD (mg mL^{-1})
Coconut shell liquid smoke	1	88.72	87.40	1.19	87.40± 1.19
	2	86.44			
	3	87.04			
Quercetin	1	11.58	12.70	1.22	12.7 ± 1.22
	2	12.51			
	3	13.99			

3.5 Antibacterial Activity Test Against *E. Coli* Bacteria

The disc diffusion method was applied to test antibacterial activity. The selection of this method was driven by the formation results of clear zones, which are more accessible to observe. This method helped determine the sensitivity of pathogenic bacteria, both aerobic and facultative anaerobic, to various antimicrobial compounds [33]. The growth medium employed Mueller-Hinton agar (MHA). WHO has recommended MHA media for antibacterial tests, predominantly aerobic and facultative anaerobic bacteria, for food and clinical materials. This media has been proven to provide good results and reproducibility [34].

This study utilized several concentrations: 40, 80, 120, 160, and 200 mg mL^{-1}. The positive control employed 10 mg/ml amoxicillin, while the negative control utilized distilled water. Amoxicillin was adopted due to its broad spectrum against gram-negative or gram-positive bacteria [35]. Table 3 discloses the antibacterial test.

Table 3 exhibits that the diameter inhibition zone for all the concentrations of coconut shell liquid smoke was under 5 mm, indicating a weak activity as antibacterial in comparison to amoxicillin, depicting medium activity as an antibacterial. The identification of coconut shell liquid smoke unveiled its flavonoid compounds. Flavonoids are the largest group of phenolic compounds that can work as antiseptics and disinfectants by denaturing and coagulating bacterial cell proteins. Flavonoids have antibacterial effects by inhibiting cell membrane function and bacterial energy metabolism. When inhibiting cell membrane function, flavonoids form extracellular protein complex compounds, which can damage the cell membrane of *E. coli* bacteria, leading to the release of intracellular compounds from the bacteria [36].

Table 3. The diameter of the inhibition zone for the antibacterial activity test.

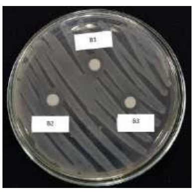
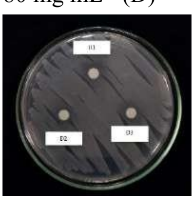
Concentration	Diameter of inhibition zone (mm)			
	Replication I	Replication II	Replication III	Average
coconut shell liquid smoke 200 mg mL^{-1} (A) 	4.55	5.20	4.80	4.85
coconut shell liquid smoke 160 mg mL^{-1} (B) 	5.05	4.50	3.70	4.42
coconut shell liquid smoke 120 mg mL^{-1} (C) 	3.80	6.20	2.65	4.22
coconut shell liquid smoke 80 mg mL^{-1} (D) 	3.20	4.10	5.00	4.10
coconut shell liquid smoke 40 mg mL^{-1} (E) 	4.00	3.10	4.50	3.87

Table 3. Continued

Concentration	Diameter of inhibition zone (mm)			
	Replication I	**Replication II**	**Replication III**	**Average**
Distilled water 	3.53	4.85	2.85	3.74
Amoxicillin 10 mg mL^{-1} 	6.38	6.15	5.60	6.04

4 Conclusion and recommendation

Coconut shell liquid smoke produced from the pyrolysis of coconut shells offers potential benefits for human life, particularly in the food industry and farming. The physical properties of coconut shell liquid included a yellow liquid with a distinctive odor, a pH of 2, and an acid content of 4.82%. The chemical content of coconut shell liquid smoke included saponins and flavonoids. The GCMS analysis disclosed phenol as the dominant compound. Coconut shell liquid smoke had antioxidant potential with an IC50 of 87.40 mg mL^{-1}, categorized as a strong antioxidant. This antioxidant activity provides benefits for food preservation. Meanwhile, coconut shell liquid smoke did not have antibacterial activity against *E. coli*. Hence, its bacterial strength in inhibiting the test bacteria remained unproven.

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