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Determination of secondary metabolite content of phenolic group from liquid smoke of coconut shell and husk (Cocos nucifera L.) using visible spectrophotometry

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ABSTRACT

So far, waste in Indonesia has been increasing. One of the types of waste commonly found in the community is coconut shell and husk waste. However, these wastes contain compounds with potential benefits for health systems. One of the beneficial compounds for health is phenolics and flavonoids. This study aims to determine the total phenolic and flavonoid content in liquid smoke from coconut shells and husks of grades 3, 2, and 1. The stages of this research include raw material processing, pyrolysis, distillation, phytochemical screening, and determination of the liquid smoke content from coconut shells and husks of grades 1, 2, and 3 using visible spectrophotometry. The results showed that the liquid smoke from coconut shells and husks contained flavonoids, tannins, saponins, triterpenoids/steroids, and glycosides. The determination of flavonoid and total phenolic content was carried out by determining the maximum wavelength of quercetin and gallic acid and calculating the total flavonoid and phenolic content using UV-Vis spectrophotometry. The results of determining the total flavonoid content in liquid smoke from coconut shells and husks of grades 1, 2, and 3 were 0.0720±0.828; 0.08975±0; 0.3002±0.00166 mgQE/mL and 0.04393±0; 0.05336±0; 0.52776±0 mgQE/mL. The results of determining the total phenolic content in liquid smoke from coconut shells and husks of grades 1, 2, and 3 were 13.425±0.0447; 14.3583±5.1691; 19.4416±0.1084 mgGAE/mL and 17.016±0.0423; 18.40±0; 18.9083±0.0423 mgGAE/mL. The highest phenolic and flavonoid content was found in the grade 3 liquid smoke, while the lowest content was found in the grade 1 liquid smoke.

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INTRODUCTION

Coconut (Cocos nucifera L.) is an agricultural product with high economic value and is widely grown in coastal areas. The utilization of coconut is not limited to just the fruit, but the entire coconut plant can be used to meet the economic, social, and cultural needs of the community and the

environment (Ahadiyat et al., 2020). Efforts to optimize coconut shells, which are waste from the coconut grating and coconut milk extraction process, have been utilized as raw material for producing liquid smoke (Restuhadi, 2015). The solid waste from liquid smoke has the potential to be developed into beneficial products with economic value, as it contains organic materials in relatively high amounts, such as lignin (36.51%), cellulose (33.61%), and hemicellulose (19.27%) (Idiawati et al., 2021). So far, the handling of coconut shell waste has not been optimal, with much of the coconut shell waste being disposed of into rivers or drains, causing flooding (Zumaro & Arbi, 2017). A method that is considered very effective and efficient for handling solid waste from coconut shells and husks is the pyrolysis method. Pyrolysis is a chemical decomposition process of organic materials through heating in the absence of oxygen, where the raw material undergoes a breakdown of its chemical structure into gas. The pyrolysis process to produce liquid smoke can be produced from wood or coconut shells at a temperature of 400 °C. Liquid smoke from materials containing hemicellulose requires pyrolysis temperatures of 220-400 °C, from cellulose at temperatures of 320-420 °C, and from lignin at temperatures above 400 °C (Fauziati et al., 2018). One of the advantages of the pyrolysis method in processing coconut shell waste is that it produces liquid smoke, activated charcoal, and methane gas (Sarwendah et al., 2019).

Liquid smoke is a product of condensation or the condensation of vapor from the direct or indirect combustion of materials containing lignin, cellulose, hemicellulose, and other carbon compounds. During pyrolysis, cellulose compounds produce carbonyls and acetic acid, along with its homologs, while lignin compounds produce phenols and tar. Hemicellulose compounds produce furfural, furan, and carboxylic acids (Suhendi, 2012). Liquid smoke has many benefits, especially in the food, fisheries, wood, and plantation industries. In the food and fisheries industries, the primary use of liquid smoke is as a preservative to extend shelf life and add aroma and flavor (Sarwendah et al., 2019). This is because liquid smoke contains antimicrobial and antioxidant compounds. In the wood industry, liquid smoke is useful for repelling termites (Evahelda et al., 2021). Additionally, liquid smoke can be used to eliminate odors in poultry farms due to its compounds that have a smoky smell, such as carbonyls, furans, phenols, cyclopentene, benzene, and others (Saputri et al., 2021).

Based on the benefits of liquid smoke from coconut shells and husks, it is necessary to determine the total phenolic and flavonoid content from the pyrolysis of liquid smoke, as phenolics in liquid smoke can be used as food preservatives. The method used for this determination is visible spectrophotometry with the addition of FeCl3, which will cause the sample to change from a greenish-brown color to a dark blue. Considering the numerous benefits derived from utilizing coconut shell and husk waste, which is processed into liquid smoke, this study is conducted. The aim of this research is to describe the process of producing liquid smoke from coconut shell and husk waste, classify the liquid smoke based on its color (grade), and measure the total phenolic and flavonoid content in liquid smoke from coconut shells and husks.

RESEARCH METHOD

The research conducted is an experimental study involving a series of stages, starting from sample collection and processing, preparation of reagent solutions, to the pyrolysis of liquid smoke and distillation of pyrolysis products. Furthermore, phytochemical screening and determination of total phenolic and flavonoid content were carried out using visible spectrophotometry. This study was conducted from February to July 2024, with research locations at the Research Laboratory of the Faculty of Pharmacy, Universitas Muslim Nusantara Al-Washliyah Medan, the Research Laboratory of the Faculty of Pharmacy, Universitas Sumatera Utara, and the Energy Conversion Laboratory, Politeknik Negeri Medan. The materials used included Aquadest, Folin Ciocalteau reagent, Na_2CO_3 20%, gallic acid, quercetin, various chemical reagents such as Mayer's, Bouchardat's, Dragendorff's, $FeCl_3$, and several other materials such as 70% ethanol, methanol, and distilled water. The equipment used included a set of distillation apparatus, UV-Vis spectrophotometer, vortex, analytical balance,

micropipettes, volumetric flasks, stir bars, and various other laboratory tools that support the research process.

In the data analysis of this study, the first step taken was phytochemical screening to determine the presence of bioactive compounds in the samples. The results of this screening were analyzed qualitatively to identify the types of compounds present, such as alkaloids, flavonoids, saponins, terpenoids, and phenolics, based on their reactions with the chemical reagents used. Next, to determine the total phenolic and flavonoid content, the data obtained from visible spectrophotometry were analyzed by measuring absorbance values at specific wavelengths. The results were compared with the calibration curve of the phenolic standard (gallic acid) and flavonoid standard (quercetin) to determine the concentration of each compound in the sample. The pyrolysis and distillation processes were also analyzed to separate and identify the components produced, evaluating the efficiency of each stage in isolating the bioactive components. If the study involved multiple treatment groups, comparisons between groups were conducted using statistical tests such as t-tests or ANOVA to determine significant differences in the concentrations of the compounds tested. Additionally, the data obtained were tested for normality using the Shapiro-Wilk test to ensure the distribution of the data, and if the data was not normal, non-parametric tests were used. The results of the analysis were presented in the form of tables or graphs to facilitate interpretation, providing an overview of the active compound content in the samples and the potential for further applications, particularly in the development of natural-based products.

RESULTS AND DISCUSSIONS

Plant Identification Results

The plant identification conducted at the Laboratory of the Faculty of Mathematics and Natural Sciences, Department of Biology, Universitas Sumatera Utara, showed that the sample used in this study was coconut (Cocos nucifera L.) from the Fabaceae family. In this study, the parts used were the coconut shells and coir.

Sample Processing Results

The samples used in this study were coconut shells and coir (Cocos nucifera L.), with sample weights of 11 kg and 8 kg, respectively. The pyrolysis process, a conversion technique involving the combustion of biomass combined with condensation, was carried out. The results of this process can be seen in the table below.

Pyrolysis and Distillation of Liquid Smoke Results

Table 1. Pyrolysis Results

No.	Raw Material (Sample)	Pyrolysis Temperature and Time	Raw Material Weight (kg)	Liquid Smoke Yield (ml)
1	Coconut Shell	400°C, 4 hours 15 minutes	11 kg	1000 ml
2	Coconut Coir	400°C, 4 hours 15 minutes	8 kg	800 ml

For the coconut shells, a liquid smoke yield of grade 3 (9.09% b/b) was obtained from 11 kg of raw coconut shell material, resulting in 1000 ml. The low yield of grade 3 liquid smoke was due to the large amount of coconut shell turning into charcoal. For grade 2, the yield was 66.66% v/v, as 900 ml of grade 3 liquid smoke was distilled, resulting in 600 ml of grade 2 liquid smoke. For grade 1, the yield was 40% v/v, with 500 ml of grade 2 liquid smoke, producing 200 ml of grade 1 liquid smoke. For the coconut coir, a liquid smoke yield of grade 3 (11.25% b/b) was obtained from 8 kg of raw coconut coir material, resulting in 900 ml. For grade 2, the yield was 62.5% v/v, as 800 ml of grade 3 liquid smoke was distilled, producing 500 ml of grade 2 liquid smoke. For grade 1, the yield was 70% v/v, with 500 ml of grade 2 liquid smoke, producing 350 ml of grade 1 liquid smoke.

Table 2. Liquid Smoke Yields

Sample	Liquid Smoke Yield
	Grade III
Coconut Shell	9.09% b/b
Coconut Coir	11.25% b/b

Liquid Smoke Characteristics Testing

The organoleptic testing of liquid smoke includes the evaluation of color and aroma. There are noticeable differences in the color and smell of liquid smoke from various grades, which are influenced by the presence of tar and carbonyl compounds in the liquid smoke. The compounds that significantly contribute to the aroma of liquid smoke are syringol, guaiacol, and ethyl guaiacol. Guaiacol and syringol are responsible for the smoky flavor and aroma (Erickson et al., 1979). Carbonyl compounds (aldehydes and ketones) mainly affect the color. The color of smoked products forms due to the interaction between carbonyl compounds and amino groups. Generally, liquid smoke has a sharp, acidic, burnt smell, similar to the scent of disinfectants or medicines.

For grade 3 liquid smoke, the high content of tar, carbonyl, and phenolic compounds predominates, resulting in a dark brown color and a smell resembling thick smoke. This meets the quality standards outlined in the Indonesian National Standard 8985:2021, where grade 3 liquid smoke is expected to be yellow to brown. In grade 2 liquid smoke, tar is absent because tar does not evaporate, with a boiling point of 495°C, so it remains in the distillation flask. However, carbonyl compounds are still present, giving the liquid a brownish hue, while phenol, which has a boiling point of 181°C, is partially reduced because the distillation temperature during purification is set at 150°C, leading to a less intense odor. In grade 1 liquid smoke, there is a significant reduction in carbonyl compounds, which is reflected in the absence of the yellow to brown color. Despite this, it still retains the characteristic flavor and aroma, as some phenolic compounds remain, though they are diminished due to the redistillation process.

Phytochemical Screening

The results of the phytochemical screening conducted on the liquid smoke from coconut shell and husk are presented in Table 3.

Table 3. Phytochemical Screening Results for Liquid Smoke from Coconut Shell and Husk

No.	Compound Group	Coconut Shell	Husk
1.	Alkaloids	-	+
	Mayer	-	+
	Dragendorff	-	+
	Bouchardat	-	+
2.	Tannins	+	+
3.	Saponins	+	+
4.	Flavonoids	+	+
5.	Steroids/Triterpenoids	+ Triterpenoid	+ Triterpenoid
6.	Glycosides	+	+

Phytochemical screening of liquid smoke from both coconut husk and shell was conducted to identify the presence of secondary metabolites. The test utilized specific reagents as detectors for certain compounds. The results showed that liquid smoke from coconut husk was positive for alkaloids, tannins, saponins, flavonoids, triterpenoids/steroids, and glycosides, while the coconut shell smoke was negative for alkaloids but positive for tannins, saponins, flavonoids, triterpenoids/steroids, and glycosides.

Alkaloids

The results indicate that the liquid smoke from coconut husk contains alkaloids. Alkaloid testing was performed using three reagents: Mayer, Dragendorff, and Bouchardat. The positive

result for alkaloids with Mayer's reagent is indicated by the formation of a white to yellowish precipitate. This occurs due to the interaction between alkaloids and tetraiodomercurate (II) ions, forming a complex that precipitates out. The mercury ion is a heavy metal ion capable of precipitating alkaline alkaloids (Svehla, 1990). For Dragendorff's reagent, alkaloids form a brick-red precipitate (Septiana et al., 2005). According to McMurry and Fay (2004), Marliana et al. (2005), and Sangi et al. (2013), alkaloid-containing compounds form an orange or reddish-brown precipitate when tested with Dragendorff's reagent, due to the interaction between alkaloids and tetraiodobismuthate (III) ions.

The positive result for Bouchardat's reagent is characterized by the formation of a brown precipitate. This precipitate forms due to the coordination bond between the K+ metal ion and alkaloids, resulting in a potassium-alkaloid complex that precipitates (Nafisah et al., 2014). The liquid smoke sample from coconut husk tested with Bouchardat's reagent formed a brownish-black precipitate, indicating the presence of alkaloids. In contrast, the coconut shell liquid smoke did not show a positive result for alkaloids. The absence of a white precipitate in Mayer's test ($HgCl_2$ + KI) indicated that there were no alkaloids present. The solution turned orange, but no precipitate formed, indicating that no alkaloid-complex was formed. Similarly, no orange precipitate formed when Dragendorff's reagent was added, and no brownish-black precipitate was observed with Bouchardat's reagent, confirming the absence of alkaloids.

Flavonoids

Flavonoids were tested for presence using magnesium (Mg) and concentrated hydrochloric acid (HCl). Upon adding Mg and HCl to the liquid smoke from both coconut shell and husk, a yellow or orange color appeared, indicating the presence of flavonoids. According to Harborne (1987), flavonoid compounds are reduced by Mg and HCl, resulting in red, yellow, or orange coloration.

Tannins

The test results showed that both the liquid smoke from coconut husk and shell contained tannins. Tannin identification was done using $FeCl_3$ reagent. The color change to dark green-black occurred due to the formation of a complex between tannins and $FeCl_3$. Tannins are polyhydroxyphenolic compounds (polyphenols) that can differentiate from other phenolic compounds due to their ability to precipitate proteins. Tannins are polar due to the OH group, and when $FeCl_3$ is added, the color changes to dark blue or green-black, indicating the presence of tannins (Suryani et al., 2023). According to other references, tannins with $FeCl_3$ undergo hydrolysis, forming a dark blue-black color.

Phenolic compounds, which contain an aromatic ring and one or more hydroxyl (OH) groups, are polar. The phytochemical test results indicated the presence of phenolic compounds in both the liquid smoke from coconut husk and shell, marked by the green-black color change.

Saponins

The test results showed that the liquid smoke from coconut husk contained saponins. Saponins are surface-active compounds easily detected by their ability to form foam. The glycosidic bonds within saponins make them polar. The positive result for saponins was confirmed by the formation of foam, 1-10 cm high, within approximately 10 minutes (Kemenkes, 2020). The screening results also showed that the liquid smoke from coconut shell contains saponins. The foam forms because saponins contain both hydrophilic (water-soluble) and hydrophobic (soluble in non-polar solvents) components, acting as surfactants that lower surface tension (Mbougueng et al., 2020). When agitated, the hydrophilic group binds with water, and the hydrophobic group binds with air, forming bubbles.

Triterpenoids/Steroids

Steroid testing was conducted using the Liebermann-Burchard test. In this test, if a red or purple color forms, it indicates the presence of triterpenoids, while a green color indicates the presence of steroids (Kemenkes, 2020). The results showed the formation of red or purple color, confirming the presence of triterpenoids in the sample.

Glycosides

The glycoside test for the liquid smoke from both coconut shell and husk involved adding sulfuric acid through the walls of a test tube. A purple ring formed between the two layers of liquid, indicating the presence of glycosides in the samples of liquid smoke from both coconut shell and husk.

Results of Maximum Wavelength Measurement for Quercetin Flavonoid

The maximum wavelength measurement for quercetin solution resulted in a value of 439 nm with an absorbance of 0.432. This data was then used to measure the absorbance and establish a calibration curve for quercetin solution as well as the samples. To obtain measurable absorption in the visible light region, flavonoids were reacted with a color-forming reagent, such as $AlCl_3$. Quercetin was chosen as the reference solution because it belongs to the flavonoid group known to react with $AlCl_3$ (Chang, 2002). The results are visualized in Figure 4.1 below.

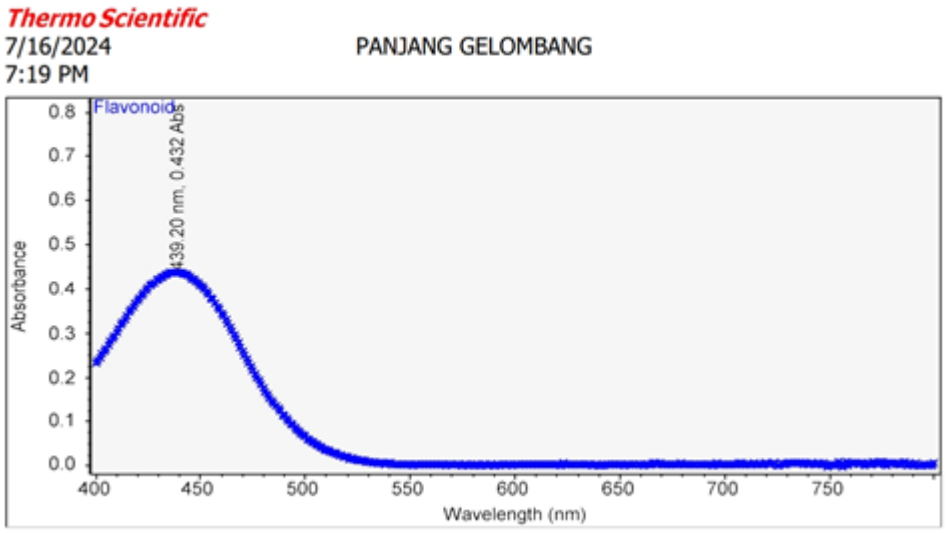

Figure 1. Maximum Wavelength of Quercetin Flavonoid

Operating Time Measurement Results

The color of the $AlCl_3$ reagent solution must be optimized by determining the correct working time for measurement because the absorbance value in visible light spectroscopy is greatly influenced by the color of the solution. The optimal working time was established by adding samples to the quercetin standard solution and measuring absorbance at wavelengths between 600 nm and 800 nm. Stable measurement results were observed at 25 minutes.

Results of Quercetin Calibration Curve

The calibration curve was constructed using various concentrations of quercetin standard solution: 2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml, and 10 µg/ml, each reacted with $AlCl_3$ reagent. Each solution was measured at a wavelength of 439 nm. The resulting calibration curve, showing the

relationship between gallic acid concentration (µg/ml) and absorbance, met the required absorbance range of 0.2 to 0.8 (Puspitasari et al., 2019). The results are provided in Table 4 below:

Table 4. Absorbance Values of Quercetin Standard Solution

Concentration (µg/ml)	Absorbance	Regression Equation
0	0.000	
2	0.123	
4	0.266	y = 0.0742x – 0.0136
6	0.435	
8	0.602	
9	0.717	

From the data in the table, the calibration curve is obtained as shown below:

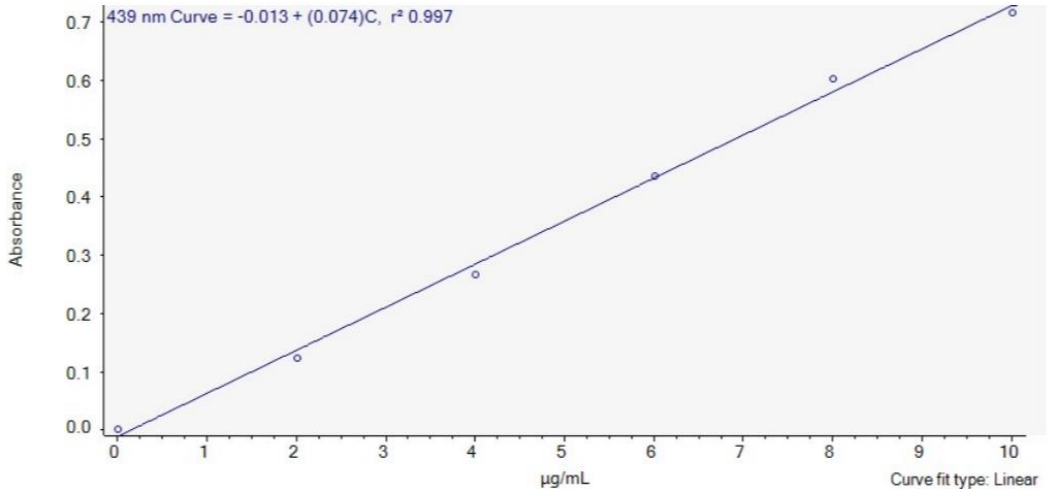

Figure 2. Quercetin Calibration Curve

The regression equation obtained for the quercetin standard solution is: **y = 0.0742x – 0.0136**, with a correlation coefficient of **0.997**. The linearity value indicates a strong correlation between concentration and the resulting absorbance.

Results of Flavonoid Content in Coconut Shell and Husk (Cocos nucifera L.) Liquid Smoke

The flavonoid content was analyzed using a spectrophotometric method. Visible spectrophotometry is an analysis that uses ultraviolet electromagnetic radiation in the range of 190-380 nm and visible light in the range of 380-780 nm. The principle of visible spectrophotometry is the interaction between matter and light of a certain wavelength (Veranita, 2022). In the measurement of total flavonoid compounds, the sample solution was treated with $AlCl_3$ to form a color complex, causing a shift in the wavelength towards the visible spectrum, as indicated by the yellow color of the solution. The addition of sodium acetate aimed to maintain the wavelength in the visible region (Veranita, 2022). The total flavonoid content was determined using the linear regression equation y = ax + b, derived from the quercetin calibration curve, to calculate the concentration (x). The obtained x value was then substituted into the total flavonoid calculation formula. The total flavonoid content was determined by repeating the process six times, and the actual content of each sample is presented in the following table:

Table 5. Average Total Flavonoid Content in Coconut Shell and Husk Liquid Smoke

Sample	Actual Total Flavonoid Content (mg QE/mL Sample)
	Grade I
Coconut Shell	0.0720 ± 0.828
Coconut Husk	0.04393 ± 0

From the results in the table, it is shown that the total flavonoid content in the coconut husk at grade III is higher compared to the coconut shell. However, in grade II and III, the total flavonoid content is higher in the coconut shell. Several factors affect the total flavonoid content in the liquid smoke samples, one of which is the pyrolysis temperature, moisture content of the raw materials, and the composition of compounds in the raw materials. The total flavonoid content in the liquid smoke from coconut shell and husk is highly influenced by the moisture content of the raw material during pyrolysis. Lower moisture content in the liquid smoke increases the concentration of active compounds such as flavonoids and phenols. Coconut shell contains more lignin compared to coconut husk, but the cellulose content is lower in coconut shell than in coconut husk (Djatmiko et al., 1985).

Results of Maximum Wavelength for Gallic Acid Phenolic Compounds

The total phenolic testing began with the measurement of the maximum wavelength of the gallic acid standard solution, which was reacted with the Folin-Ciocalteu reagent to produce a blue color. This can be measured using visible spectrophotometry with a concentration of 300 µg/ml, yielding a maximum wavelength of 729 nm with an absorbance of 0.202. According to Gandjar and Rohman (2007), the complementary color for phenolic testing is bluish-green, which corresponds to the wavelength range of 600-800 nm. The result can be seen in Figure 3 below.

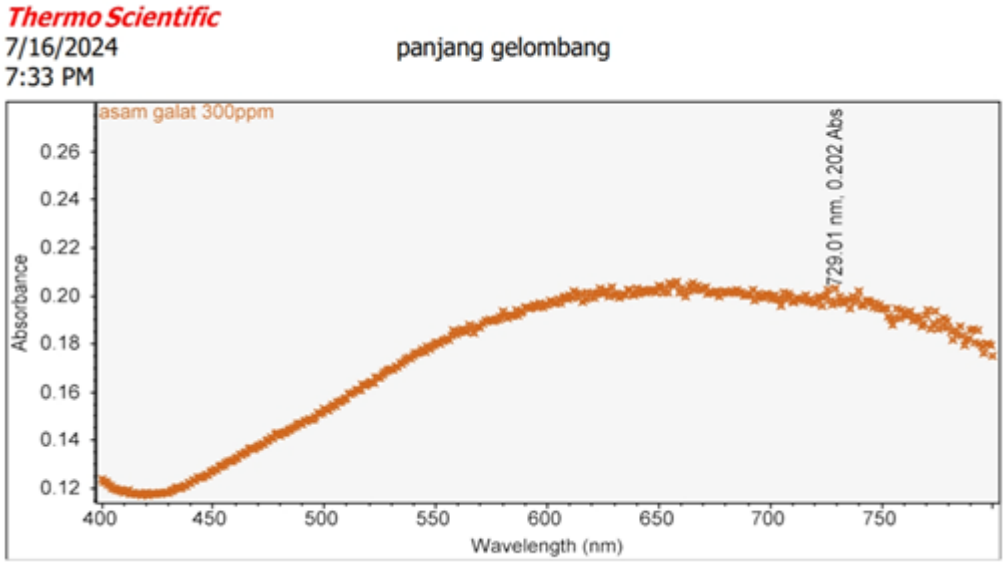

Figure 3. Maximum Wavelength of Gallic Acid Phenolic Compounds

Results of Operating Time Measurement

The color of the Folin-Ciocalteu reagent standard solution tends to be unstable, so it is necessary to determine the optimal working time for measurement, as absorbance in visible spectrophotometry is significantly influenced by color. The optimal working time was determined by using the Folin-Ciocalteu reagent standard solution, followed by the addition of samples, and measuring the absorbance at wavelengths between 600-800 nm.

Results of Gallic Acid Calibration Curve

A calibration curve was created with various concentrations of gallic acid standard solutions: 200 µg/ml, 300 µg/ml, 400 µg/ml, 500 µg/ml, and 600 µg/ml, all reacted with the Folin-Ciocalteu reagent. Each solution was measured at a wavelength of 729 nm, resulting in a calibration curve between the gallic acid concentration (µg/ml) and absorbance, which met the required range of 0.2 - 0.8 (Puspitasari et al., 2019). The results are presented in Table 6 below:

Table 6. Absorbance Values of Gallic Acid Standard Solution

Concentration (µg/ml)	Absorbance	Regression Equation
0	0.000	
200	0.141	
300	0.200	y = 0.001x - 0.001
400	0.253	
500	0.325	
600	0.415	

From the data in the table above, the calibration curve is shown in the figure below:

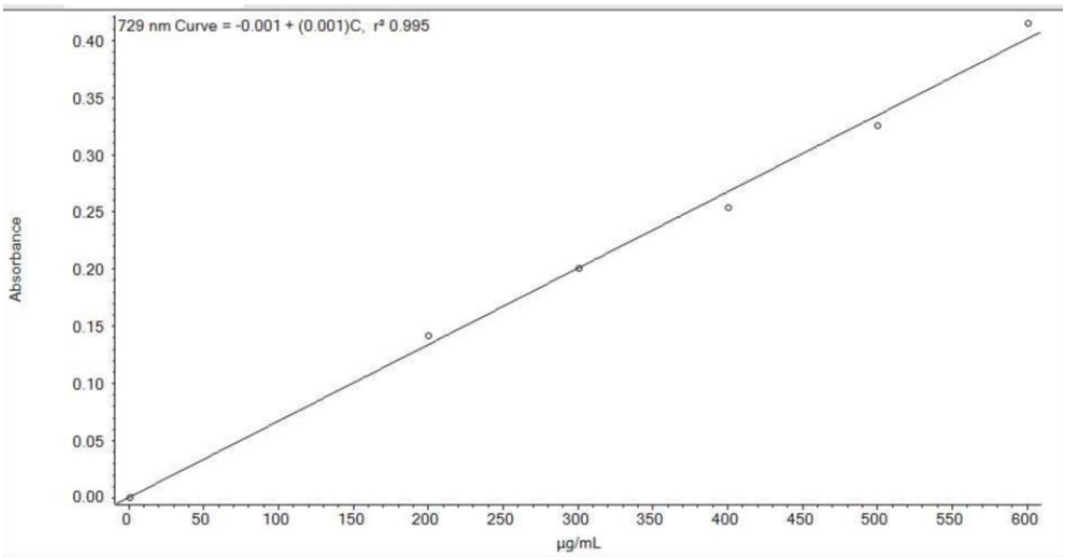

Figure 4. Gallic Acid Calibration Curve

The regression equation obtained from the gallic acid standard solution is y = 0.001x - 0.001 with a correlation coefficient of 0.995. The linearity value demonstrates a strong correlation between the concentration and absorbance.

Results of Total Phenolic Content in Liquid Smoke from Coconut Shell and Coir (Cocos nucifera L.)

The total phenolic content in liquid smoke from coconut shell and coir was determined using UV-Vis spectrophotometry. Gallic acid, a stable and natural phenolic compound, was used as the standard. Gallic acid is a hydroxybenzoic acid derivative and a simple phenolic acid. It reacts with the Folin-Ciocalteu reagent, resulting in a yellow color, indicating the presence of phenolic compounds. Sodium carbonate (Na_2CO_3) was then added to create a basic environment, facilitating the reduction of Folin-Ciocalteu by hydroxyl groups in the phenolic compounds of the sample (Lingbeck et al., 2014). The total phenolic content was calculated using the linear regression equation y = ax + b, derived from the gallic acid calibration curve, to obtain the concentration (x). This value was then substituted into the formula for calculating the total phenolic content. The total phenolic

content was determined through six repetitions, and the actual content for each sample is presented in the table below:

Table 7. Actual Total Phenolic Content in Liquid Smoke from Coconut Shell and Coir

Sample	Total Phenolic Content (mg GAE/mL Sample)
	Grade I
Coconut Shell	13.425 ± 0.0447
Coir	17.016 ± 0.0423

The results indicate that the total phenolic content in Grade III liquid smoke from coconut shell is higher than that from coir. This is because coconut shell contains more lignin compared to coir, while its cellulose content is lower. For Grade II and III, the total phenolic content is strongly influenced by the distillation temperature of the liquid smoke and its water content, which affects the concentration of active compounds such as phenols (Djatmiko et al., 1985).

CONCLUSION

Based on the phytochemical screening results, the liquid smoke derived from coconut shell contains several bioactive compounds, including flavonoids, tannins, saponins, steroids/triterpenoids, and glycosides. Meanwhile, the phytochemical screening of liquid smoke from coconut husk shows the presence of alkaloids, flavonoids, tannins, saponins, steroids/triterpenoids, and glycosides. The total flavonoid content in the liquid smoke from coconut shell for grades I, II, and III is 0.0720±0.828, 0.08975±0, and 0.3002±0.001 mg QE/mL sample, respectively. In contrast, the total flavonoid content in the liquid smoke from coconut husk for grades I, II, and III is 0.04393±0, 0.05336±0, and 0.52776±0 mg QE/mL sample. Additionally, the average total phenolic content in the liquid smoke from coconut shell for grades I, II, and III is 13.425±0.0447, 14.3583±5.1691, and 19.4416±0.1084 mg GAE/mL sample, respectively. For coconut husk, the total phenolic content for grades I, II, and III is 17.016±0.0423, 18.40±0, and 18.9083±0.0423 mg GAE/mL sample.

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