Indonesian Journal of Chemical Research

http://ojs3.unpatti.ac.id/index.php/ijcr Indo. J. Chem. Res., 12 (2), 145-152, 2024

Analysis of Chemical Components and Antioxidant Activity in Nutmeg Shell Liquid Smoke Processed through Rotary Evaporator Purification

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Received: June 2024
Received in revised: August 2024
Accepted: September 2024
Available online: September 2024

Abstract

This study aims to analyze liquid smoke's chemical content and antioxidant activity from pyrolysis of nutmeg (Myristica fragrans) shell with rotary evaporator purification. A rotary evaporator purified liquid smoke from the pyrolysis of nutmeg shells. Identification of the chemical components of liquid smoke from the pyrolysis of nutmeg shells was carried out by Gas Chromatography-Mass Spectroscopy (GC-MS), and Antioxidants were measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method with absorbance at a wavelength of λ 517 nm. The results of GC-MS analysis showed 14 peaks and 19 types of compounds detected with the 2-methylphenol compound component located at a retention time of 4.974 minutes, standing out with the largest contribution in terms of area, reaching 47.74%. The antioxidant test showed that the nutmeg shell has IC50 values before and after rotary evaporation of 0.40% and 0.07%, having strong antioxidant activity, and the results at a concentration of 3.2 mg/ml provided a level of free radical protection of more than 50%, namely 91.14%. The main compounds that play as antioxidants are phenol compounds and their derivatives, such as 2-methoxy-phenol and 2-methoxy-4-methyl-phenol.

Keywords: Nutmeg shell, Liquid smoke, GC-MS, DPPH, Antioxidant

INTRODUCTION

Nutmeg (*Myristica fragrans*, Houtt) is one type of plant found in North Sulawesi. One of the most popular agricultural products in North Sulawesi is nutmeg. Nutmeg shells are considered waste because they are made from nutmeg seed shells (Dareda et al., 2020), and consist of fruit flesh (77.8 percent), mace (4 percent), shells (5.1 percent), and seeds (13.1 percent) from nutmeg seeds (Launda et al., 2017). Nutmeg shells are one of the wastes from nutmeg seed processing, which has great potential to be used as raw material for making liquid smoke, activated charcoal, and smokers that are very easy to find (Salindeho et al., 2017). Vinegar acid, also known as vinegar, is made through the dry distillation process of smoking raw materials, such as wood. The condensation process occurs in a water-cooled condenser.

The results of several studies show that the chemical content of liquid smoke, especially phenol compounds, can act as an antioxidant. In addition, some critical studies that support this include: 1) Finding the Coconut Shell Liquid Smoke Extract's Antimicrobial Composition: Based on earlier research demonstrating the strong antibacterial activities of phenols and other chemicals in liquid smoke, this study discovered the presence of antibacterial substances in coconut shell liquid smoke (Lombok et al., 2014). 2) Potential Use of Liquid Smoke Products from Pyrolysis of Nutmeg Seed Shells as Fumigation The results show that many phenol compounds in this liquid smoke have strong antibacterial and antioxidant properties (Salindeho. N et al., 2018). 3) GC-MS Analysis and Antioxidant Activity of Liquid Smoke from Nutmeg Shell Waste: This study used GC-MS to identify chemical components in the liquid smoke from nutmeg seed shells and evaluate their antioxidant activity. The results show that this liquid smoke contains many phenol compounds that have high antioxidant and antibacterial activity (Tjakra et al., 2022).

Numerous chemicals, such as vitamin C, tocopherol, flavonoids, carotenoids, and other phenolic compounds, can be exogenous antioxidants. These can all protect people against diseases linked to

oxidative stress. It's critical to find new, natural antioxidants from food crops. This is so that these naturally occurring antioxidants can protect the body against a range of disorders caused by oxidative damage to lipids, proteins, and nucleic acids.

One of the main reasons nutmeg shells were chosen for this inquiry was the lack of thorough studies examining at their antioxidant capability. The active phytochemicals found in nutmeg shells, such as lignans, flavonoids, carotenoids, terpenoids, vitamins, phenolics, and alkaloids, are responsible for these antioxidant levels. Their multiple antioxidant roles include removing metals, removing radicals, stopping lipid peroxidation, and chilling single oxygen molecules.

With the above background in mind, the purpose of this experiment was to use rotational purification to determine the evaporator's chemical analysis and antioxidant activity.

METHODOLOGY

Tools and Materials

Pyrolysis tool. GC-MS Agilent Technologies 7890 Gas Chromatograph with Auto Sampler, 5975 Mass Selective Detector and Chemstation data system, Rotary evaporator.

Karegesan village is in North Minahasa Regency, where nutmeg shells are taken. Methanol pro analysis (Brand), ascorbic acid pro analysis (Merck), and 2,2-diphenyl-2-picrylhydrazil/DPPH (Aldrich) are the chemicals used.

Procedure

Manufacture of liquid smoke from pyrolysis of nutmeg shells (ACHPCP)

After the dried nutmeg shells are crushed with a hammer to obtain smaller samples, a pyrolysis process occurs and liquid smoke is generated. The method (Sersermudy et al., 2020) is used to perform this procedure. This method uses a pyrolysis furnace equipped with an LPG stove as the reactor and heater. This furnace is 37.5 cm in diameter and 50 cm high and can hold 8 kg of raw materials. Look at the temperature. Pyrolysis occurs for four to five hours a temperature of 300-400°C. Next, the smoked liquid is kept for seven days at room temperature to remove sediment and supernatants. Cast deposits separate sediments and supernatants from the smoked liquid and produce filtrates from the smoked liquid. After the purification process through the rotary evaporator, the product is stored at room temperature in bottles.

Liquid Smoke Purification Process by Rotary Evaporator distillation method

The rotary evaporator distillation process is used to purify the liquid smoke products. One method of purifying liquid smoke based on the evaporation principle is the rotavapor method. This procedure involves heating the liquid smoke from pyrolysis to a temperature of at least 70 °C in a revolving reactor. The volatile ingredients in the liquid smoke will evaporate and rise to the top of the reactor as the temperature and pressure inside the reactor rise. Steam condenses more quickly into a liquid because of the freezing effect of the reactor's top-level cold air. The liquid smoke is then purified by using a condenser to separate it from the air.

GC-MS test for ACHPCP chemical content

Analysis of liquid smoke before and after purification was identified using HP Ultra 2 columns with a length of 30 m, diameter of 0.20 mm LD, and film thickness of 0.11 μm using GC-MS Agilent Technologies 7890 Gas Chromatograph with Auto Sampler and 5975 Mass Selective Detector and Chemstation data systems. With gas transporting helium. The split technique for separation injection uses a spill ratio of 100:1 and an injection volume of 3 μL. The ionization temperature of the injector reaches 230°C and 250°C for one minute and then increases by 20°C/min to 280°C for twenty-six minutes. Temperatures start at 70 °C with 3 °C/min to 150 °C. To find the components of the resulting compound, you can compare the spectral data obtained with the mass spectrum from the Gas Chromatography-Mass Spectrometry (GC-MS) database at the NIST Library (Hasan et al., 2024).

Antioxidant Test (DPPH Method)

Preparation of DPPH Stock Solution 500 ppm 100 ml

Weigh 50 mg DPPH and put it in a 100 ml measuring flask. After that, dissolve with methanol until the limit mark of a 100 ml flask appears. (Lung & Destiani, 2017).

Preparation of DPPH 50 ppm 100 ml test solution

Pipette 10 ml of DPPH solution 500 ppm and put it into a 100 mL measuring flask, then add methanol to 100 ml. Next, measure absorption at a wavelength of 517 nm (Maesaroh et al., 2018).

Manufacture of Vitamin C Solution 100 ppm 100 ml

Weighing as much as 10 mg of ascorbic acid pro analysis (Merck) dissolved with methanol into a measuring flask to 100 mL (Purwanto et al., 2017).

Antioxidant activity of vitamin C by DPPH method (comparison)

Pipettes of 100 µL, 200 µL, 400 µL, 800 µL, and 1600 µL of 100 ppm Vitamin C solution. Then, add 2 mL of 50 ppm DPPH and methanol to the test tube until the volume is 5 mL (Hasanela et al., 2023). Measure absorption and absorbance with a spectrophotometer at a wavelength of 517 nm after letting stand for 30 minutes at room temperature in a dark place (Shahidi & Zhong, 2015). To create a calibration curve, plot the concentration of vitamin C and absorbance. Then, calculate the linear regression value. The calculation of the percentage of absorption inhibition of DPPH IC_{50} can be used to determine the antioxidant activity of vitamin C, which is indicated by the magnitude of DPPH radical uptake inhibition.

Antioxidant activity of ACHPCP by DPPH method (sample)

To measure antioxidant activity, each test solution was pipettes of 0.02 mL, 0.04 mL. 0.08 mL, 0.16 mL, and 0.32 mL. Then, add 2 mL DPPH 50 ppm and add methanol up to a volume of 5 mL in a test tube. Measure the absorption with UV–VIS spectrophotometry at a wavelength of 517 nm after properly combining the mixture and leaving for 30 minutes in a dark place (Fitri et al., 2023). After plotting the absorbance and concentration of the sample, create a calibration curve and find its linear regression value (Riyandari & Multazam, 2023)

Data analysis

The calculation of the percentage of DPPH absorption inhibition can be used to determine the antioxidant activity of the sample based on the absorption inhibition of DPPH radicals. (Merghem & Dahamna, 2020):

$$\%\text{Inhibisi} = \frac{\text{Abs. B} - \text{Abs. S}}{\text{Abs. B}} X\ 100\% \qquad (1)$$

Information:
Abs. B = DPPH absorbant 50 µM
Abs. S = Absorbance Test Sample

The IC_{50} value, or 50% inhibitory concentration (Taufiq & Sulfiani, 2023), is used to measure antioxidant activity. This value that indicates the concentration of a test solution that can inhibit radical activity by 50% (Fitriani et al., 2020). The linear regression equation formula calculates the IC_{50} value for each sample concentration. This formula shows the relationship between the concentration of the antioxidant fraction, represented by the x-axis, and the percent inhibition, represented by the y-axis of the measurement replication series (Nintiasari, J & Ramadhani, M.A, 2022).

RESULTS AND DISCUSSION

The results of observations on the liquid smoke of nutmeg shells before the pyrolysis process are displayed in Figure 1 point A. Liquid smoke now seems solid brown in appearance. It becomes evident that the liquid and tar smoke are not the same after a week when a tar precipitate forms on the bottom of the container. A rotary evaporator is used to carry out an extra purification step that results in purer liquid smoke, as seen in Figure 1 Point B. While liquid smoke products are of higher quality, this purification process is meant to eliminate tar and other impurities.

A Pyrolysis liquid smoke

B Liquid smoke after rotary evaporator

Figure 1. Liquid smoke from pyrolysis of nutmeg and rotary evaporator results

Results from Gas Chromatography-Mass Spectrometry (GC-MS) analysis.

Before the rotary evaporator is used, nutmeg shells undergo pyrolysis, as shown by the findings of the GC chromatogram on liquid smoke in Figure 2. Figure 2 shows a chromatogram of liquid smoke produced from the pyrolysis of nutmeg shells before the rotational evaporator. In retention time (t_R), the highest peaks were 4,119 minutes (area 4.36%), 4.939 minutes (area 17.76%), 5.256 minutes (area 6.33%), 5.380 minutes (area 5.25%), 7.525 minutes (area 10.15%), 9.711 minutes (area 4.99%), and 10.200 minutes (area 10.18%).

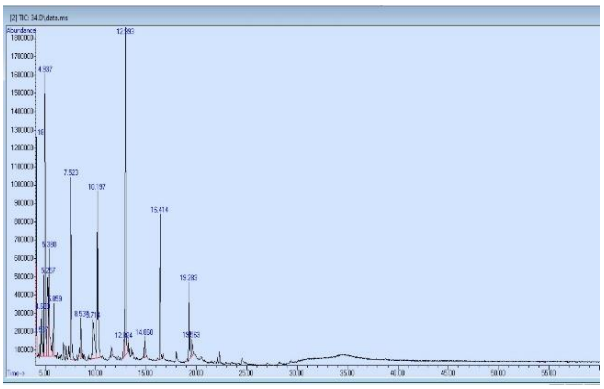


Figure 2. Pyrolysis Liquid Smoke GC Chromatogram (before rotary evaporator)

Following the rotary evaporator, nutmeg shells undergo pyrolysis to generate liquid smoke, as seen in the chromatogram presented in Figure 3. Retention time (t_R) measures five prominent peaks: 4.684 minutes (area 4.53%), 4.974 minutes (area 47.74%), 5.277 minutes (area 4.02%), 7.559 minutes (area 23.96%), and 10.207 minutes (area 9.10). These peaks only indicate that the nine primary chemicals are carriers.

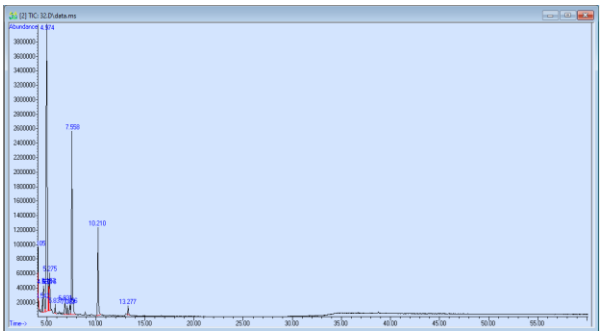


Figure 3. Liquid Smoke GC Chromatogram Pyrolysis results (after rotary evaporator)

The compound's chemical formula, as displayed in Figure 4, is $C_7H_8O_2$. Thus, the detected compound's molecular structure is comparable to 2-methoxy-phenol (guaiacol), which is recorded in the database.

Figure 4 shows the mass spectrum of 2-methoxy phenol found in the GC-MS database (NIST. LIB, entry no. 231011 A), which has a formula mass of 124 and molecular formula $C_7H_8O_2$. The mass spectrum of this compound was found at t_R 4.974 min of liquid smoke produced from the pyrolysis of nutmeg shells. Based on Figure 4, the compound 2-methoxy-phenol has a molecular mass of 124 g/mol. The mass spectrum has M^+ at m/z 124, which indicates the mass of the compound formula, the base peak at m/z 109 and m+1 at m/z 125 with low intensity.

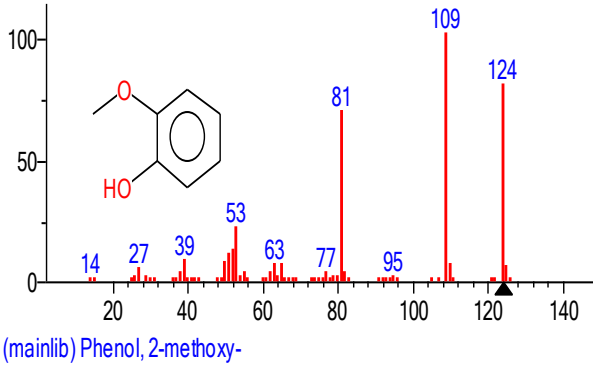


Figure 4. Mass spectrum of compounds at retention time (t_R) 4.974 minutes from ACHPCP

The mass spectrum pattern shows that fragments of compounds produce ions at m/z: 109, 95, 81, 65, 53, and 39. In the fragmentation of the compound, there is a radical release of the methyl group [M^+ -CH_3] so that ions are formed at m / z 109 [$C_6H_5O_2$] $^+$, which is relatively stable and is the base peak of the mass spectrum of the compound. From the m/z 109 ion, there is a release of carbon monoxide radicals [-CO] and carbon dioxide [-CO_2] forming ions at m/z: 81 [C_5H_5O]$^+$, and m/z 65 [C_5H_5]$^+$, followed by the release of carbon monoxide radicals [-CO] forming ions at m/z 53 [C_4H_5]$^+$, followed by the release of methylene radicals [-CH_2] forming ions at m/z 39 [C_3H_3]$^+$ (Moldoveanu, S.C, 2019).

It was determined that the chemical at t_R 4.974 minutes of liquid smoke from the pyrolysis of nutmeg shells was 2-methoxy-phenol based on the collected spectrum data and the description of the compound beheading pattern. The mass spectrum of compounds is suggested to be decapitated as shown in Figure 5.

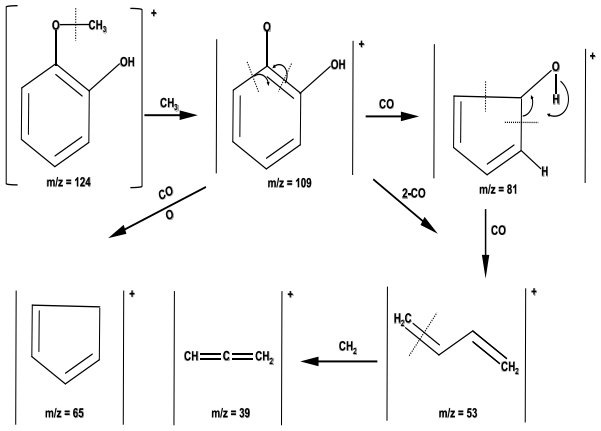


Figure 5. Fragmentation of 2-methoxy-phenol at a retention time (t_R) of 4.974 minutes from ACHPCP

Gas chromatographic (GC) analysis is a very useful technique in identifying chemical compounds in complex mixtures. In this case, a peak in retention time (t_R) of 7.559 minutes with an area of 23.96% signifies the presence of a particular compound in the analyzed sample. With reference to the available databases, the identifying of the compound as 2-methoxy-4-methylphenol (creosol) can be confirmed. The conformity of the mass spectrum obtained with reference data for creosol provides additional support to this identification.

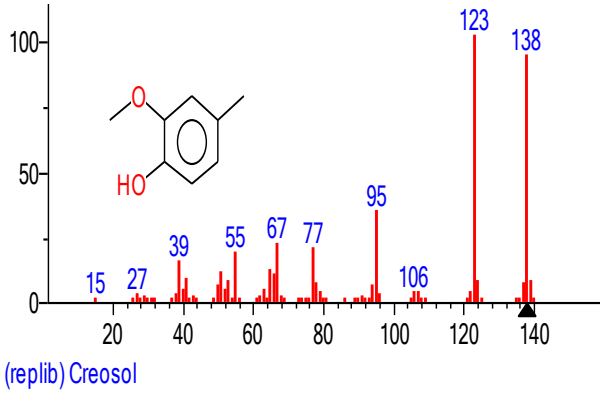


Figure 6. Mass spectrum of compounds at retention time (t_R) 7.559 minutes of ACHPCP

The mass spectrum of 2-methoxy-4-methyl-phenol found in the GC-MS database (NIST. LIB, entry number 231011 A) is similar to the spectrum found in liquid smoke produced from pyrolysis of nutmeg shells at tR 7.559 min (Figure 6). The molecular formula $C_8H_{10}O_2$ and the mass of the formula 138 are the same.

In the fragmentation of the compound, there is a radical release of the methyl group [M^+-CH_3] so that ions are formed at m/z 123 $[C_7H_7O_2]^+$, followed by the release of radicals [.CO] forming ions at m/z 95 $[C_6H_7O_]^+$. From the m/z 95 ion it is suspected that there are 3 kinds of possible radical release, namely radical [. C_3H_4], [.CO] and [H_2O.]. Radical release [. C_3H_4] produces ions with m/z 55 $[C_3H_3O]^+$ followed by radical release [.CO] and [H_2O.] yielding ions at m/z 67 $[C_5H_7]^+$ and m/z 77 $[C_6H_5]^+$. From the m/z 67 ion is also released the C_2H_4 radical forms an ion at m/z 39 $[C_3H_3]^+$.

Based on the resulting spectral data and the description of the beheading of the compound, it was concluded that the compound at t_R 7.559 minutes of liquid smoke from pyrolysis of nutmeg shells was 2-methoxy-3-methyl-phenol. A pattern of fragmentation of the mass spectrum of compounds is proposed, as presented in Figure 7.

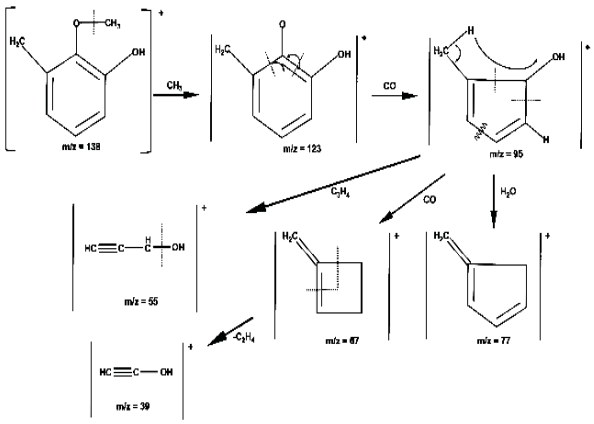
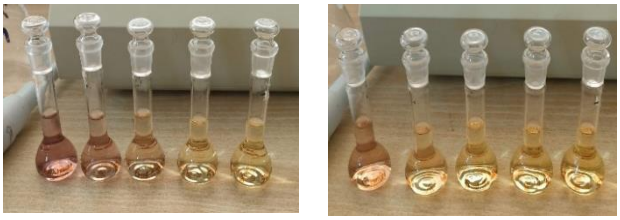


Figure 7. Fragmentation of 2-methoxy-3-methyl-phenol compound at retention time (t_R) 7.559 min from ACHPCP

Antioxidant test with DPPH method

this study shows that the liquid smoke from the pyrolysis of nutmeg shells has significant antioxidant activity as indicated by the color change from purple to yellow and a decrease in absorbance at a wavelength of 517 nm. The active compounds in this liquid smoke can capture and deactivate free radicals, which suggesting that they can serve as effective and powerful antioxidant agents.



a. Antioxidants against the concentration of liquid smoke from pyrolysis of nutmeg shells before rotary evaporator

b. Antioxidants against the concentration of liquid smoke from pyrolysis of nutmeg shells after rotary evaporator

Figure 8. Antioxidant Test Liquid smoke from pyrolysis of nutmeg shells

Figure 8 displays the percentage of liquid smoke antioxidants from the pyrolysis of nutmeg shells (after the rotary evaporator) and test results of the DPPH free radical fighting power of liquid smoke (before the rotary evaporator). The liquid smoke produced by the pyrolysis of nutmeg shells exhibits strong antioxidant action, as evidenced by the decrease in absorbance at a wavelength of 517 nm. The absorbance is declining in this test.

The reactions that take place using the DPPH method are referred to as redox reactions (reduction and oxidation). It is composed of the two distinct reactions, reduction (which gains electrons) and oxidation (which removes electrons). In Figure 9, redox reactions are displayed.

Because it is stabilized by delocalization of the aromatic ring, DPPH is a stable radical that can easily trap other radicals but does not undergo dimerization. DPPH *Free Radical Scavenging Assay* solution is dark purple when reduced to reaction with hydrogen donor. This happens due to strong absorption bands at wavelengths of about 517 nm. Then it becomes colorless until it becomes pale

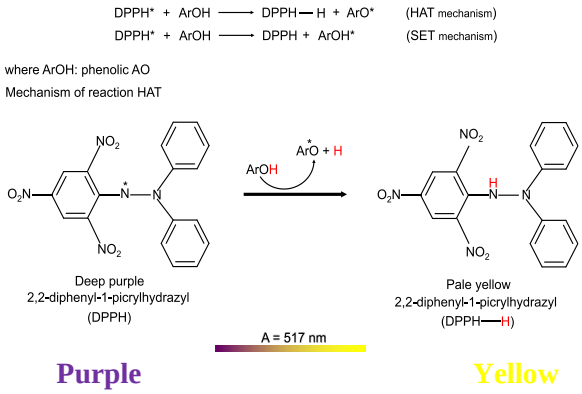


Figure 9. Antioxidant chemical reactions (Sari & Herdyastuti, 2024).

yellow. The decrease in absorbance is linearly correlated with antioxidant concentrations.

Table 1. Results % Inhibition and IC_{50} various concentrations of ACHPCP (before rotary evaporator)

Concentration (%)	Absorbance	IC_{50}
0.2	0.464	
0.4	0.403	
0.8	0.303	10.81
1.6	0.178	
3.2	0.028	

Table 1 and Figure 10 show the antioxidant capacity of liquid smoke from the pyrolysis of nutmeg shells and its main components. The liquid smoke from the pyrolysis of nutmeg shells before the rotary evaporator has IC_{50} at a concentration of 10.81 ppm, which shows strong antioxidant activity.

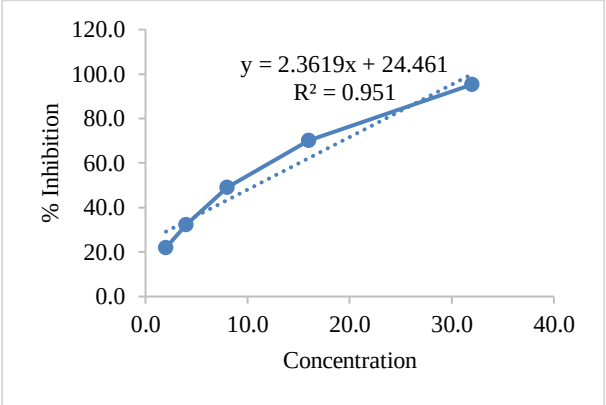


Figure 10. Graph of the % linear regression equation Inhibition of various concentrations of Vitamin C

Figure 11 and Table 2 display the antioxidant potential and primary ingredients of the liquid smoke produced by pyrolyzing nutmeg shells. Liquid smoke from pyrolyzing nutmeg shells before the rotary evaporator exhibits high antioxidant action, as demonstrated by its IC_{50} concentration of 0.40 ppm.

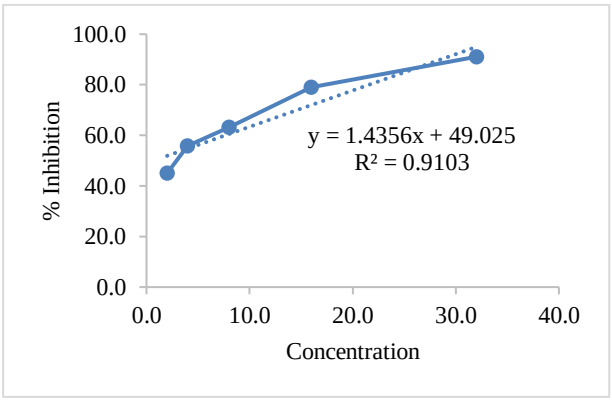


Figure 11. Graph of the linear regression equation % Inhibition of various concentrations of ACHPCP (before rotary evaporator)

Table 2. Results % Inhibition and IC_{50} various concentrations of ACHPCP (before rotary evaporator)

Concentration (%)	Absorbance	IC_{50} (ppm)
0.2	0.275	
0.4	0.239	
0.8	0.195	0.40
1.6	0.120	
3.2	0.042	

The outcomes demonstrated that nutmeg shell pyrolysis can yield liquid smoke, with good antioxidant properties. This liquid smoke has a considerable anti-free radical effect even at relatively modest doses.

The liquid smoke generated by pyrolyzing nutmeg shells exhibits notable antioxidant activity, as demonstrated by a decrease in absorbance at a wavelength of 517 nm in Tables 1 and 2. The response between the antioxidant molecules in the liquid smoke and the DPPH free radicals in this test is demonstrated by the decrease in absorbance, which effectively lowers the quantity of active free radicals.

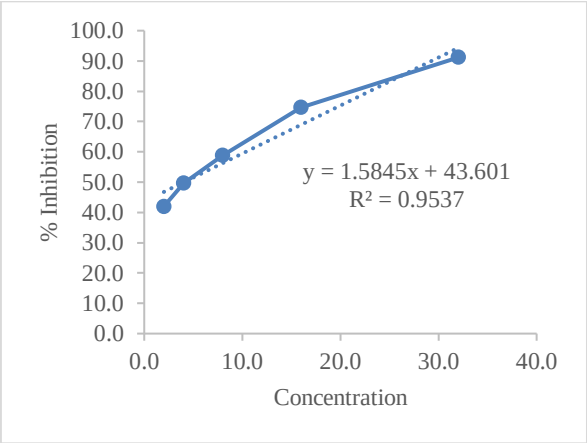


Figure 12. Graph of the linear regression equation % Inhibition of various concentrations of ACHPCP (after rotary evaporator)

The antioxidant capacity of liquid smoke derived from the pyrolysis of nutmeg shells, and its principal constituents are distinctly displayed in Figure 12 and Table 3. The liquid smoke had an IC_{50} of 0.07 ppm after the rotary evaporator operation, indicating a significant level of antioxidant activity. Consequently, it can be said that there is a great chance that the liquid smoke made from nutmeg shell pyrolysis will be useful as an antioxidant.

Table 3. Results % Inhibition and IC_{50} various concentrations of ACHPCP (after rotary evaporator)

Concentration (%)	Absorbance	IC_{50} (ppm)
0.2	0,275	
0.4	0,221	
0.8	0.184	0.07
1.6	0.105	
3.2	0.045	

The DPPH method relies on the reaction of capturing hydrogen atoms from antioxidant compounds by DPPH. This results in IC_{50}, which is very strong at <50 ppm, very strong at 50-100 ppm, medium at 101-150 ppm, and weak at >150 ppm. (Wibowo et al., 2018).

CONCLUSION

GC-MS analysis showed that 2-methoxy-phenol (Guaiacol) from 17.76% before rotary evaporator to 47.74% after rotary evaporator, as well as 2-methoxy-4-methyl-phenol (Creosol) from 10.15% to 23.96% after rotary evaporator. With an IC_{50} < 50 ppm, namely 0.40 ppm before and 0.07 ppm after the rotary evaporator, the DPPH free radical scavenging study of liquid smoke from nutmeg shell pyrolysis (ACHPCP) demonstrated extremely significant antioxidant activity.

ACKNOWLEDGMENTS

The author thanks the DKI Jakarta Health Laboratory, the Technical Laboratory, the Medical Laboratory, Health Polytechnic, the Ministry of Health, Manado, and the Laboratory of the Faculty of Mathematics, Natural, and Earth Sciences, Manado State University for helping and playing an important role in the implementation of the research conducted.

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