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Analysis Antioxidant IC₅₀ Liquid Smoke of Cocoa Skin with Several Purification Methods

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Abstract. Cocoa skin waste can be used as the raw material for making liquid smoke by pyrolysis. Liquid smoke has a function as the preservative in food. The use of liquid smoke as the preservative must go through several purification processes. The purpose of this study was to determine the antioxidant content of cocoa skin liquid smoke in several purification methods. The purification process uses several methods in purifying liquid smoke, such as distillation, purification with zeolite, purification by active charcoal and the mixture of zeolite and active charcoal. The results of IC₅₀ antioxidant activity of cocoa skin liquid smoke by distillation, purification with zeolite, purification with active charcoal and combined purification between active charcoal and zeolite obtained IC₅₀ values of 217.55 ppm for the distillation method, 164.15 ppm for purification with active charcoal and zeolite, 143.43 ppm for purification with zeolite and 44.05 ppm for purification with active charcoal. Purification with active charcoal is the best treatment and has a very strong category of Antioxidants compared to the other three treatments.

Keywords: IC₅₀ Antioxidant, Cocoa Skin, Liquid Smoke, Purification

1. Introduction

Cocoa skin is one of the agricultural wastes that its utilization is still very limited. Cocoa farmers usually only discard and let the skin of the cocoa skin rot and dry around the cocoa plantations. Cocoa skin has many benefits if further processed. Some reports mention the content of cocoa skins have active compounds that are beneficial to humans. Cocoa skins contain phenolic compounds and flavonoids [1]. The polyphenol content includes cinnamic acid, tannin, pyrogallol, quercetin, resorcinol, and epicatechin-3-galat [2]. Previous studies have reported that cocoa skins have antibacterial compounds to inhibit bacterial growth, including research by [3] that extracts bioactive components from cocoa skin waste and their effects on antioxidant and antimicrobial activities, [4] conducted antibacterial activity tests of cocoa skin extracts (*Theobroma cocoa* L.) against *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus*, [5] analyzing the chemical substance of cocoa skin liquid smoke with GC-MS method. Seeing the potential of the cocoa skin, this study utilizes the cocoa skin to produce liquid smoke.

Liquid smoke is a result of pyrolysis or condensation of vapor from indirect or direct combustion of materials that contain lots of carbon and other compounds. Raw materials that are widely used are wood,



palm oil humps, pulp produced by wood, and others. According to [6], liquid smoke is compounds that evaporate simultaneously from heat reactors through pyrolysis techniques and condensed in the cooling system.

Liquid smoke contains various compounds that can be grouped into groups of phenol compounds, acids, and carbonyl compound groups. Groups of these compounds play the role of the antimicrobial, antioxidant, flavoring, and coloring. Because liquid smoke can act as an antimicrobial and antioxidant, liquid smoke can be used as a preservative [7]. Antioxidants are compounds that can inhibit oxidation reactions, by binding to free radicals and highly reactive molecules. As a result, cell damage can be inhibited [8]. Based on the source, antioxidants can be classified into 2 types, namely natural antioxidants and antioxidant synthesis. However, there are concerns about the side effects of synthetic antioxidants making natural antioxidants the chosen alternative [9].

The antioxidant activity of liquid smoke from rubber fruit shells can be determined from the inhibitor concentration value of 50% (IC₅₀). IC₅₀ is a value that indicates the concentration of the sample that can inhibit free radical activity by 50%. Each of these values and the percent inhibition of known concentration plotted on the graph so obtained linear equation $y = mx + c$ to change the value of y with 50 of linear line equation, then it will get the value of x that becomes the value of IC₅₀. IC₅₀ values obtained from the straight-line equation of 101.27 ppm. The smaller IC₅₀ value, the stronger the antioxidant activity of the compound. A compound is said to be a very strong antioxidant if IC₅₀ value is less than 50 ppm, active if it is worth 50-100 ppm, while if it is 101-250 ppm and weak if it is worth 250-500 ppm. The obtained IC₅₀ values can indicate that the liquid smoke of rubber fruit shells has moderate antioxidant activity [10].

Research on the antioxidant effect of liquid smoke is very important considering that liquid smoke is currently being used commercially by the food industry [11]. Purification of liquid smoke has been carried out by [12] using active charcoal to purify the liquid smoke of durian skin. Furthermore [13] purifies liquid smoke from a coconut shell using a filtration method with active charcoal and zeolite and distillation. Research by [14], which purifies liquid smoke with different types of active charcoal. Research by [15], purifies liquid smoke by the distillation method.

Based on the description above, this research was conducted the purification of smoke liquid by several methods and analyze the value of antioxidants IC₅₀ liquid smoke cocoa skin with some methods of purification. The purpose of this method is to determine the results of the antioxidant activity of IC₅₀ liquid smoke of cocoa skin by distillation, purification with zeolite, purification with activated charcoal and a combination of zeolite and activated charcoal, so that it can be used as a natural preservative in food.

2. Materials and Methods

This research was conducted at the Agricultural Product Technology Laboratory of Ekasakti University in July - September 2019. This study uses an exploratory design by looking at the IC₅₀ content antioxidant activity of liquid smoke with several purification methods. Liquid smoke can be obtained by the pyrolysis method.

The material used in this study was cocoa skin obtained from Lubuk Minturun Village, Padang City, and pyrolysis was carried out until liquid smoke of cocoa skins was obtained. Aquades, active charcoal, zeolite, methanol (p.a), diphenyl pycril hidrazil (DPPH) (Sigma Aldrich).

The equipment used is a series of pyrolysis tools, distillation equipment series, 250 ml filter, test tubes, gels cups, glass funnels, filter paper, micropipettes, vortex, plastic wrap, aluminum foil and photometer Spectro.

2.1 Purification of liquid smoke

2.1.1. Distillation [13]

This process is carried out to separate components from a mixture on the basis that some components can evaporate faster than other components. The resulting steam contains more volatile components, so the process of separating the components from the mixture can occur. Distillation smoke liquid can be carried out at a temperature of 100oC to 150oC. The liquid smoke distillation process can also eliminate unwanted compounds, namely tar compounds and aromatic polycyclic hydrocarbons.

2.1.2. Active charcoal [16]

The liquid smoke filtrate from active zeolite filtration is refiltrated using active charcoal derived from activated cocoa shell charcoal. The liquid smoke filtrate is put into a column containing activated carbon then the filtrate obtained is collected in a container for testing.

2.1.3. Zeolites [17]

Liquid smoke resulting from filtering is then filtered using active zeolite by flowing distillate liquid smoke into the active zeolite column to obtain the purified liquid smoke filtrate using zeolite. The filtrate is stored in a container for testing.

2.1.4. Mix of active charcoal and modified zeolite [17]

Liquid smoke produced from the pyrolysis process is added to active charcoal and zeolite, then allowed to stand for overnight. After 24 hours the liquid smoke is filtered using filter paper, then the value of its antioxidant activity is observed.

2.2. Testing of IC₅₀ Antioxidant Activity [18]

2.2.1. Sample preparation

Liquid smoke samples used for the antioxidant activity test were taken from the filtering results with several purification methods, namely with active charcoal, purification with zeolite, purification by distillation and purification with a mixture of active charcoal and zeolite. Weigh the sample up to 0.1 gr plus 10 ml of methanol (pa) as mother liquor. Samples from the mother liquor are weighed and made in various concentrations, namely: 15, 30, 45, 60, and 75 ppm into 15 test tubes. Pipette 1 ml solution of each concentration plus 2 ml of methanol and 1 ml dpph then let stand for 30 minutes, then read using a UV-VIS spectrophotometer at a wavelength of 517 nm. As a blank, 3 ml of methanol was used and added with 1 ml dpph.

2.2.2. Testing IC₅₀ antioxidant activity of liquid smoke

The method used in determining the antioxidant activity of liquid smoke of cocoa skin is using the DPPH method (2,2-diphenyl-1-pikrihidrazil). DPPH method used DPPH (2,2-diphenyl-1-pikrihidrazil) as free radicals. This method was chosen because the method is simple, fast, and easy for screening the radical capture activity of several compounds, besides this method has proven to be accurate and practical [19] The measured absorbency of DPPH is the absorbance of DPPH that remains after being reacted with the test solution. From the residual DPPH absorbance value, it can be seen the antioxidant activity of each test solution in inhibiting DPPH free radicals. From the absorbance value obtained then calculated percent inhibition (damping) against DPPH free radicals, namely the amount of activity of antioxidant compounds that can capture DPPH free radicals. Calculation of percent DPPH antioxidant activity used the following formula:

$$\text{Antioxidant activity (\%)} = \frac{(A \text{ blank} - A \text{ sample})}{A \text{ blank}} \frac{(A \text{ blank} - A \text{ sample})}{A \text{ blank}} \times 100\%$$

Information:

A blank = radical DPPH absorption of 0.4 mM

A sample = radical DPPH 0.4 mM uptake after sample treatment.

The amount of antioxidant activity from cocoa skin smoke liquid can be determined from the inhibitor concentration values of 50% (IC50). IC50 is the value that indicates the concentration of the sample that can inhibit radical activity by 50%. Each of the inhibition % value and known concentration plotted on the graph so obtained linear equation $y = mx + c$ to change the value of y with 50 of linear line equation, it will get the value of x as the value of IC50.

3. Results and Discussion

Liquid smoke used in this study is liquid smoke from the skin of cocoa fruit taken from the pyrolysis without purification (grade 3). In this study, it carryout the purification of cocoa skin smoke liquid by using several methods of purification, such as purification with active charcoal, with zeolite, with active charcoal + zeolite and distillation. In testing the antioxidant activity, it employs DPPH radical compounds as indicators to see the percentage of free radical inhibition. This method is commonly used because it has simple advantages and the work is carried out very simply. Measurement of the antioxidant activity of the sample is done by looking at the wavelength path which is the maximum wave DPPH.

The presence of antioxidant activity from the sample results in a change in color in the DPPH solution in methanol which was originally dark purple to brownish-yellow. This is a change in color or what is called reacting. The results of the antioxidant activity test at all concentrations obtained must be measured using a UV-VIS spectrophotometer. Antioxidants activity is indicated by the value IC50 (Inhibitory Concentration 50%). The IC50 value is the value of the concentration of antioxidants to reduce 50% of free radical activity. The test results a percentage inhibition of antioxidant using several methods of purification of liquid smoke in Table 1 and the linear equation antioxidant activity or inhibition of free radicals using the DPPH method, these can be seen in Figures 1, 2, 3 and Figure 4.

Table 1. Percentage data on antioxidant inhibition of cocoa skin liquid smoke after purification.

Treatment	Solution concentration (ppm)	Abs	Blank	%inhibition
Distillation	15	0,358	0,526	17,8
	30	0,328	0,526	20,8
	45	0,306	0,526	23,0
	60	0,282	0,526	25,4
	75	0,263	0,526	27,3
Mix	15	0,356	0,526	18,0
	30	0,334	0,526	20,2
	45	0,301	0,526	23,5
	60	0,266	0,526	27,0
	75	0,226	0,526	31,0
Zeolites	15	0,159	0,526	37,7
	30	0,146	0,526	39,0
	45	0,133	0,526	40,3
	60	0,117	0,526	41,9
	75	0,101	0,526	43,5
Active Charcoal	15	0,062	0,526	47,4
	30	0,046	0,526	49,0

45	0,035	0,526	50,1
60	0,022	0,526	51,4
75	0,011	0,526	52,5

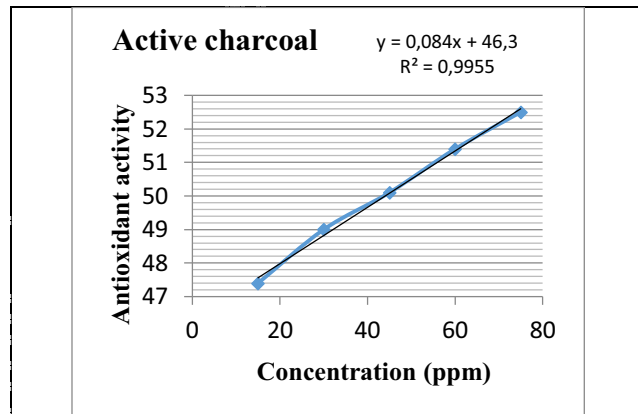


Figure 1. Antioxidant test curve of liquid smoke from active charcoal filtering

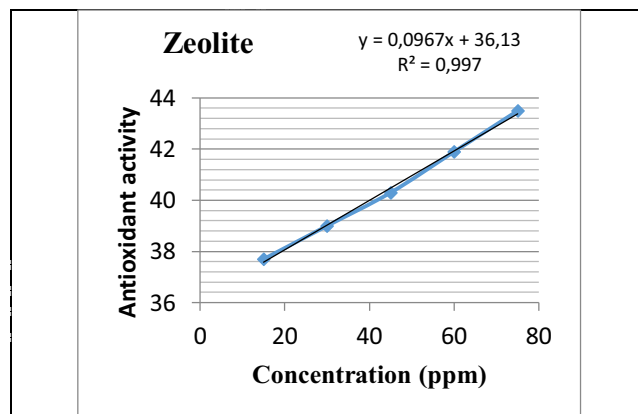


Figure 2. Antioxidant test curve of liquid smoke from zeolite filtering

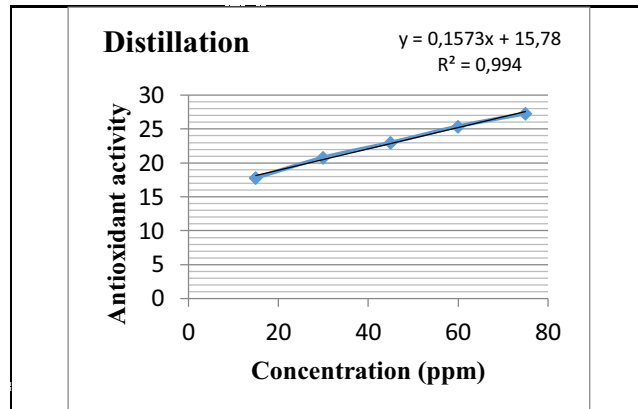


Figure 3. Antioxidant test curve of liquid smoke from Distillation

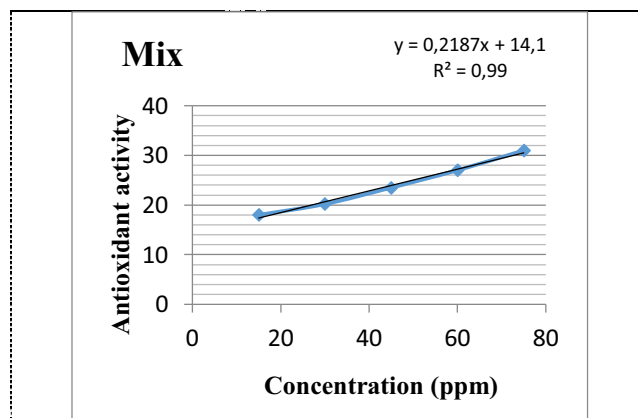


Figure 4. Antioxidant test curve of liquid smoke from active charcoal and zeolite filtering

From the curves above, then it can be calculated the value of IC₅₀ with each inhibition percent value and known concentration is plotted on the graph so obtained linear equation $y = mx + c$ to change the value of y with 50 of linear line equation, it will get the value of x as the value of IC₅₀. Furthermore, the results of probit analysis are compared with the level of antioxidant power to classify into the strength category in counteracting free radicals. IC₅₀ value calculation data is contained in Table 2.

Tabel 2. IC₅₀ values of cocoa skin liquid smoke with different purification methods

Treatment	Linear equation	a	b	y	x (ppm)	Information
Distillation	$y = 0,1573x + 15,78$	0,1573	15,78	50	217,55	intermediate
Mix	$y = 0,2187x + 14,1$	0,2187	14,1	50	164,15	intermediate
Zeolites	$y = 0,0967x + 36,13$	0,0967	36,13	50	143,43	intermediate
Active charcoal	$y = 0,084x + 46,3$	0,084	46,3	50	44,05	very strong

Based on the data in the table above, IC50 values are obtained from each purification method. Value of antioxidant activity IC50 ranging between 44.05 ppm - 217.55 ppm. The highest value is in the purification treatment using active charcoal with IC50 antioxidant activity value of 44.05 ppm and classified into a very strong category, while the lowest value is in the purification treatment using distillation with an antioxidant activity value of 215.55 ppm and classified into the medium category. The antioxidant activity test by the DPPH method was stated with IC50 (Inhibition Concentration), which is the concentration of the extract or sample that inhibited DPPH activity by 50%. The smaller IC50 value means a higher antioxidant activity. IC50 values <50-100 indicate highly active antioxidants, IC50 values <101-250 indicate moderate antioxidant activity, and IC50 values <250-500 indicate weak antioxidants, and IC50 values <500 less active as antioxidants [20].

The antioxidant activity value of IC50 liquid smoke of cocoa skin with purification using activated charcoal has the highest IC50 value due to the absorption of activated charcoal. Absorption is determined by the surface area of the particles and this ability can be higher if the charcoal is activated by active factor chemicals or by heating at high temperatures. Thus, the charcoal will experience changes in physical and chemical properties. Such charcoal is referred to as active charcoal. The absorption of active charcoal is very large, which is 25-100 % of active charcoal weight [14]. According to [12], there is a tendency to decrease the pH value of liquid smoke from pyrolysis with the purification results with active charcoal. This is due to the content of acetic acid and phenol compounds which continue to increase during the purification process. The higher the total phenol content in liquid smoke, the lower the pH value or acidic. This is directly proportional to the increase in antioxidant activity of cocoa skin liquid smoke.

Purification by using active zeolites can separate compounds that are not part of the antioxidant group such as benzopyrene so that purified liquid smoke has higher antioxidant content. This is consistent with the results of research [13], the use of active zeolite aims to obtain liquid smoke which is completely free of harmful substances such as benzopyrene. The use of zeolites is very effective in absorbing the content of benzo(a)pyrene in liquid smoke. Zeolites function as filters because they have smaller pore holes than tar and benzo (a) pyrene compounds.

Whereas the distillation process only separates compounds based on their volatile properties. It is consistent with the statement of [21], this process is done to separate the components of a mixture by using the basis that some of the components may evaporate faster than the other components. The resulting steam contains more volatile components, so the process of separating the components from the mixture can occur. Besides that, the temperature used in the distillation process is also high so that the components of antioxidant compounds will be damaged during the distillation process. According to [16], the temperature of distillation used in the purification of liquid smoke that is 100°C – 150°C.

4. Conclusions

Based on the results of the research, cocoa skin liquid smoke that is purified using active charcoal has the value of antioxidant activity IC50 by 44.05 ppm compared with the three other purifications. This value entered into antioxidant activity is very strong because if the IC50 value is less or equal to 50 ppm then it is classified as having very strong antioxidant activity, while the other three purification methods are classified as having moderate antioxidant activity.

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