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A PRELIMINARY STUDY OF THE UTILIZATION OF LIQUID SMOKE FROM PALM KERNEL SHELLS FOR ORGANIC MOUTHWASH

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ABSTRACT: Palm kernel shells are a waste product of the palm oil industry. They have a high content of cellulose, hemicellulose, and lignin, all of which can be converted through a pyrolysis process into liquid smoke that contains the oxidized organic compounds, ketones, aldehydes, phenols, and carboxylic acid groups with antimicrobial properties. The objective of this research is to study the potential use of grade 1 liquid smoke as organic mouthwash. This research considers liquid smoke’s antimicrobial properties and focuses on analyzing its ability to inhibit the growth of streptococcus mutans living in the oral cavity. The liquid smoke used was produced by pyrolysis performed in temperatures of 340-420ºC. In order to obtain grade 1 liquid smoke, distillation was subsequently carried out at 190ºC. The ability of the produced liquid smoke to inhibit streptococcus mutans was then tested for its minimum inhibitory concentration and diameter of inhibitory region. Both minimum inhibitory concentration and diameter of inhibitory region tests were performed with a liquid smoke concentration of 0.005-0.1 mg/ml. Test results of the minimum inhibitory concentration showed that the liquid smoke possesses good antimicrobial properties against streptococcus mutans, while test results of the diameter of inhibitory region was medium at 6-9 mm. The highest diameter of inhibitory region was obtained from liquid smoke created from pyrolysis performed at 400ºC and a concentrate of 0.1 mg/ml.

Keywords: Palm Kernel Shells, Liquid Smoke, Streptococcus Mutans, Mouthwash

1. INTRODUCTION

Liquid smoke is a liquid created from smoke condensation during the pyrolysis process on wooden materials that have cellulose, hemicelluloses, and lignin. These three components are needed to create a high-quality of liquid smoke; furthermore, different compositions of these compounds alter the quality of the liquid smoke produced [1]-[3]. Palm kernel shells (PKS) contain 27.7% cellulose, 21.6% hemicelluloses, and 44% lignin [1].

Haji [4] studied the liquid smoke composition gained from PKS; his studies showed a liquid smoke yield of 52.02%. Another research was conducted by Kim et al.[5], and found that the liquid smoke composition produced in a temperature of 490ºC contained around 22.1% phenol, 5.46% acetic acid, and 20% comprises other compounds. This composition indicates that PKS have the potential to be developed into high-quality liquid smoke.

Liquid smoke possesses antibacterial and antioxidant properties that may be utilized in various fields, such as medical and food industries. Previous research found that it contains effective antibacterial and antioxidant properties which inhibit and kill bacteria [6]-[8]. Research on liquid smoke conducted in the medical field was carried out by Utami [9], where it was used to treat scabies in goats. Other research found that the antimicrobial and antioxidant properties came from its content of phenol [10]. Liquid smoke was used as a natural biopesticide to inhibit the growth of *colleototrichium capsici* in chili plants [11]. In pharmaceutical and cosmetic industries, it is used mostly as skin ointment and additional ingredients in cosmetics [7],[10].

Commercial mouthwash products commonly contain alcohol as their active ingredient, providing antibacterial properties while giving clean and fresh sensations to the users [12]. However, the alcohol content may cause negative effects; for example, the potential to cause cancer increases when the alcohol concentration is above 25% [13]. Therefore, there is a need to conduct studies on non-alcoholic mouthwashes. The antimicrobial compounds found in liquid smoke have the potential to be developed as organic mouthwash. These antimicrobial properties show promise in inhibiting the growth of bacteria living in oral cavities.

This research aims to study the potential use of liquid smoke as an organic mouthwash and focuses on analyzing its ability to inhibit *streptococcus mutans* living in the oral cavity.

2. METHODOLOGY

Samples of PKS were obtained from PTPN Cot Girek, North Aceh. Pyrolysis of PKS into liquid smoke was performed in a pyrolysis reactor, in batches exposed to temperatures of 340-420ºC. The reactor was composed of a form of stainless steel and was equipped with a temperature control. More detailed explanations into the procedures of creating liquid smoke may be found in previous research [14]. The smoke was condensed using a condensation unit made of stainless steel, resulting in a grade 3 liquid smoke, which subsequently was distilled to a grade 1 in a temperature of 190ºC. The chemical compounds were then identified using Pyro-GCMS (GCMS-QP2010, SHIMADZU) based on a method developed by Guillen and Ibargoitia [15],[16]. The antibacterial activity test was performed by determining the liquid smoke's minimum inhibitory concentration (MIC) value against streptococcus mutans, using the contact method on a nutrient broth (NB) medium. In addition, the diameter of inhibitory region (DIR) was also tested as a reference to compare the antimicrobial ability between substances [17]. MIC and DIR tests were performed upon the liquid smoke with concentration of 0.005-0.1 mg/ml.

3. RESULTS AND DISCUSSIONS

3.1 Composition of Compounds in the Liquid Smoke

The composition of liquid smoke is one parameter that determines the quality of that same liquid smoke. Liquid smoke commonly contains phenol, carboxylic acid, furan, lactone, and alcohol. Different raw materials will produce different compositions [11]. The analysis results of liquid smoke produced at 420ºC are shown in table 1. In addition to the materials used, the pyrolysis temperature also helps to determine the variety of chemical components. Liquid smoke produced from PKS at a temperature of 490ºC and with 0.64 mm samples contained 22.1% phenol and 5.46% acetic acid [5]. The liquid smoke produced in pyrolysis of a fruit stem at 500ºC contains around 12.42% w/w phenol and formaldehyde, as well as around 0.2-2.0% char [2].

Tabel 1 Composition of liquid smoke produced at 420ºC (after distillation)

No.	R. Time	Area	%	Name
1	2,864	8187797	0.16	Carbamic acid, monoammonium salt (CAS) Ammonium carbamate
2	3,289	3958610	0.08	2-Propanone (CAS) Acetone
3	5,639	9.5E+08	18.39	Formic acid, methyl ester (CAS) Methyl formate
4	6,144	2.9E+08	5.64	Hexanoic acid, 5-oxo-, methyl ester (CAS) Methyl 5-Ketohexanoate
5	8,005	2.4E+08	4.64	Acetic acid (CAS) Ethylic acid
6	9,425	1.9E+07	0.36	2-Furancarboxaldehyde (CAS) Furfural
7	9,872	1.8E+07	0.35	Butanoic acid (CAS) n-Butyric acid
8	10,108	2210756	0.04	Butanoic acid (CAS) n-Butyric acid
9	0.257	5458099	0.11	2-Pentanone, 5-(1,2-propadienyloxy)- (CAS) 5-Propadienyoxy-2-Pentanone
10	10,996	2.2E+07	0.43	2-Butenal, 2-ethenyl- (CAS) Crotonaldehyde, 2-vinyl-
11	11,549	5.8E+07	1.13	2(3H)-Furanone, dihydro- (CAS) Butyrolactone
12	12,419	8.5E+08	16.47	Benzenamine (CAS) Aniline
13	12,625	7.5E+08	14.57	2,4,6-Cycloheptatrien-1-one, 2-hydroxy- (CAS) Tropolone
14	13,260	2.1E+08	4.00	Phenol, 2-methyl- (CAS) o-Cresol
15	13,603	3.9E+08	7.53	Phenol, 2-methoxy- (CAS) Guaiacol
16	14,478	9.2E+07	1.80	Phenol, 2,4-dimethyl- (CAS) 2,4-Xylenol
17	14,810	9.6E+07	1.88	2-Methoxy-4-methylphenol
18	15,208	1.8E+07	0.36	Phenol, 2,6-dimethyl- (CAS) 1-Hydroxy-2,6-dimethylbenzene
19	15,477	4.3E+07	0.84	2-Propenoic acid, 2-methyl-, ethyl ester (CAS) Ethyl methacrylate
20	15,712	8.4E+07	1.64	2,5-Dimethoxytoluene
21	16,005	6.9E+07	1.34	3-Methoxy-pyrocatechol
22	16,513	1.1E+08	2.19	Phenol, 2,6-dimethoxy- (CAS) 2,6-Dimethoxyphenol
23	17,325	2.7E+07	0.52	Benzene, 1,2,3-trimethoxy- (CAS) 1,2,3-Trimethoxybenzene (CAS) Methylsyringol
24	17,692	6907259	0.13	1,3-Dithiolane, 2-(28-norurs-12-en-17-yl)- (CAS)
25	17,992	6284551	0.12	Benzene, 1,2,3-trimethoxy-5-methyl- (CAS) Toluene, 3,4,5-trimethoxy-

3.2 MIC Value of Liquid Smoke From PKS Against Streptococcus Mutans

Mouthwash is used in medicines to prevent caries caused by bacterial activity. Caries often lead to tooth cavities and thus the disturbance of health. The bacteria that play the main role in forming caries are streptococcus mutans, which decrease pH, which prevents tooth mineralization [18],[19].

Streptococcus mutans live in tooth and gum surfaces are the main bacteria behind caries [12]. The research conducted on germ-free animals showed that the plaque comprising streptococcus mutans leads to the formation of caries [20]. Streptococcus mutans living in the oral cavity produced acid from carbohydrate remains on tooth and gum surfaces. This research tested the MIC of liquid smoke against streptococcus mutans.

An MIC value refers to the minimum concentration of an antimicrobial substance that inhibits the growth of microorganisms overnight. MIC is widely used in laboratory analyses to study the resistance of certain bacteria against antimicrobial substances. This value indicates the lowest concentration of the antimicrobial substance in which bacteria can grow [21]. MIC is also frequently used to determine the in vitro activities of new antimicrobial substances. MIC values cannot be used as a direct comparison between the ability of one antimicrobial substance to another.

Table 2 shows that the growth of streptococcus mutans started to be inhibited at a concentration of 0.02 mg/ml. This means that the liquid smoke's MIC value against streptococcus mutans was 0.02 mg/ml.

Table 2 MIC values for different pyrolysis temperatures and liquid smoke concentrations

Liquid Smoke Temperature	MIC (mg/ml)		
	0.005	0.01	0.02
340°C	-	-	+
360°C	-	-	+
380°C	-	-	+
400°C	-	-	+
420°C	-	-	+

At a concentration of 0.005 mg/ml to 0.01 mg/ml, there was no inhibitory area seen around the paper disk after a drop of the liquid smoke. The preliminary study focused, from necessity, on these MIC values to see whether the bacteria were immune to a certain antimicrobial substance. Bacteria are categorized as sensitive to a substance if MIC value is below 8 mg/ml [22]. The MIC values in table 2 are the same at every temperature and indicate that the liquid smoke has good antimicrobial properties against streptococcus mutans residing in oral cavities, due to its active compounds: acetic acid and phenol.

3.3 The Antimicrobial Properties of Liquid Smoke from PKS Against Streptococcus Mutans

A substance's antimicrobial properties are categorized as low if its DIR is less than 5 mm, meaning that its inhibitory action is weak. A DIR value of 5-10 mm signifies medium antimicrobial properties, whereas 10-19 mm is considered strong and 20 mm or more is categorized as very strong [17]. Figure 1 shows the effects of various liquid smoke concentrations on DIR values against streptococcus mutans. As shown in the picture, DIR values and liquid smoke concentrations increase in parallel, in which DIR values of 6-9 mm signified that the liquid smoke possesses medium antimicrobial properties against streptococcus mutans at a concentration of 0.005-0.1 mg/ml.

The liquid smoke's DIR value is highly influenced by its phenol and acetic acid contents. Phenol and acetic acid influence a liquid smoke's antimicrobial ability, as demonstrated by the DIR at every temperature. The post-distillation phenol concentration produced in this research was approximately 9.88-15.8%. The liquid smoke produced at different temperatures contained different compositions of phenol and acetic acid. Phenol compound can inhibit the growth of bacterial population by extending its lag-phase proportionally inside a body or a product, whereas the speed of growth in its exponential phase remained the same except with a high concentration of phenol [23].

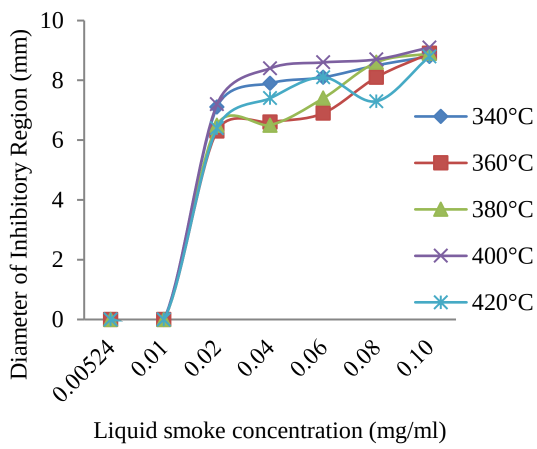

Fig. 1 The effect of liquid smoke concentration on the DIR against streptococcus mutans

The above characteristics led to different DIR values for each liquid smoke concentration. The optimum condition in which to produce liquid smoke with effective antimicrobial activity against streptococcus mutans was achieved in pyrolysis conducted in a temperature of 400ºC, which produced a liquid smoke concentration of 0.02 mg/ml, resulting in phenol and acetic acid contents of 14.15% and 22.64%.

4. CONCLUSIONS

MIC and DIR tests showed that the liquid smoke has effective antimicrobial properties against streptococcus mutans. MIC values obtained were 0.02 mg/ml constant at every temperature, which indicates that the growth of streptococcus mutans started to be inhibited at a concentrate of 0.02 mg/ml. Therefore, the temperature at which the pyrolysis is performed has no significant influence on the MIC value. However, the pyrolysis temperature and the liquid smoke concentration significantly influenced DIR value, in which the DIR increase was parallel to the liquid smoke concentration. At 0.005-0.1 mg/ml of concentration, the DIR became approximately 6-9 mm, indicating that the liquid smoke's ability to act as an antimicrobial agent against streptococcus mutans has a medium value.

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