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In Vitro Antibacterial Activity and Gas Chromatography–Mass Spectrometry Analysis of Liquid Smoke of Rice Husk

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Abstract

Liquid smoke of rice husk is one of the byproducts of the biochar manufacturing process. One of its applications in the world of food production is its potential as an antibacterial. This study aims to scientifically determine the content of the main active compounds of liquid smoke of rice husk and its potential in antibacterial activity. Five liquid smoke of rice husk samples were collected from different production batches. The antibacterial activity testing was carried out at the Pharmacology laboratory of Faculty of Veterinary Medicine UGM, while the identification of active compounds with the GC-MS test was carried out at the organic chemistry laboratory, Faculty of Mathematics and Natural Sciences UGM. Antibacterial activity test was carried out by the planting disk method and diffusion method by making wells on agar media. Based on the results of tests with GC-MS it is known that the main active compounds are Acetic acid at 25.06%, Cyclopropane, 1,1-dibromo-2-chloro-2-fluoro- at 8.58%, Cyclohexanol, 1-ethynyl-, carbamate at 6.64% and 2-Propanone at 6.07%. These compounds in biological activity have the ability to inhibit the growth of gram-positive and negative bacteria. The antibacterial activity test with the diffusion method showed a real bacterial inhibition zone with a mean of 3.62 mm from all test samples. Based on the results of this study indicate that liquid smoke of rice husk has the ability as an antibacterial and has the potential to be developed further as a natural disinfectant.

Keywords: antibacterial; GC-MS; liquid smoke; natural disinfectant; rice husk

Introduction

Liquid smoke is a result of distillation or condensation of vapor resulting from indirect or direct combustion from materials that contain a lot of carbon and other compounds. Raw materials that are widely used to make liquid smoke are coconut shells, wood, palm oil cobs, sawdust pulp, and other biomass. Liquid smoke can also mean the result of cooling and melting smoke from biomass material that is burned in a closed tube. Smoke which was originally a solid particle is cooled and then becomes a liquid is called by the name of liquid smoke. Liquid smoke is generally used as a substitute for conventional fogging techniques. The smoke composition is influenced by various factors, including wood type, wood moisture content, and combustion temperature used. The type of wood undergoing pyrolysis determines the composition of the smoke. Hardwood generally has a different composition from softwood. Hardwood is the most commonly used material because the pyrolysis of hardwood will produce a superior aroma, richer in the content of aromatic compounds and acidic compounds compared to softwood or wood containing resin.

Smoked foods, especially meat and fish, have been used as a preservation technique for centuries. Nowadays, fogging methods often involve the use of wood smoke condensate, commonly known as liquid smoke. Liquid smoke is produced by condensation of wood smoke created by pyrolysis of sawdust or wood chips followed by the removal of carcinogenic polyaromatic hydrocarbons. The main products of wood pyrolysis are phenols, carbonyl, and organic acids which are responsible for the taste, color, and antibacterial properties of liquid smoke. Some foodborne pathogens such as *Listeria monocytogenes, Salmonella, Escherichia coli* and *Staphylococcus sp* pathogens have shown sensitivity to liquid smoke in vitro and in the food system. Therefore liquid smoke has the potential to be used as a natural antimicrobial in commercial applications where the taste of smoke is desired. This review will cover the application and effectiveness of liquid smoke and liquid smoke fractions as natural food preservatives. This review will benefit the industrial and research communities in the field of food science and technology (Akharume et al., 2019; Desvita et al., 2020; Ekonomou et al., 2020; Lingbeck et al., 2014)"container-title":"Journal of Food Engineering","page":"51-57","volume":"246","source":"ScienceDirect","abstract":"The influence of the addition of Refined Liquid Smoke (RLS).

The characteristics and bioactivity of liquid smoke produced from rice husk as an antifeedant activity against larvae of *Spodoptera exigua* continue to be studied. The rice husk carbonation process is carried out to a low heating level (100-450^{o}C) and the organic component in liquid smoke is analyzed to determine the main composition. Bioassay from *S. exigua* was carried out using liquid smoke and its fractions to determine antifeedant activity. The antifeedant activity of *S. exigua* in the liquid smoke fraction increased significantly with increasing carbonation process temperatures. It is clear that the liquid smoke fraction at 250-350^{o}C is the most effective as an antifeedant against *S. exigua*. In addition, two main toxic compounds, namely, phenolic and acidic are highly concentrated in this fraction. We suggest that the compound might be a factor in the antifeedant activity of *S. exigua* larvae (Prianto et al., 2018). Antibacterial test results found that liquid smoke from redistillation showed better antibacterial activity compared to low-grade liquid smoke, whereas liquid smoke absorbed by activated carbon had the smallest activity (Fatimah, 2012).

Liquid smoke produced today is very abundant and not sold in large quantities. The information from several producers in East Java complained that their liquid smoke was not absorbed in the market. Generally, liquid smoke is only used as a pesticide and as an ingredient for preserving smoked fish where it is used very little. Farmers still rely heavily on chemical pesticides. Information circulating only states that liquid smoke has content that can kill germs and is able to eliminate the cage odor. But there is no scientific information or data that supports the statement of information in the field. Through this research, researchers want to uncover scientifically about the potential liquid smoke of rice husk as a natural disinfectant. This research will begin by analyzing the active compounds in it and prove it to be antibacterial in vitro. The right concentration is needed so that this liquid smoke is effectively used as a natural disinfectant.

Disinfectant is a basic requirement for chicken farmers, especially broilers (broilers). This relates to sanitary enclosures, hygiene of equipment and livestock production facilities. Sometimes being clean and free of disease agents is a mandatory requirement for chicken farmers before DOC (day old chick) enters the cage. The presence of disease agents is very threatening to health and can interfere with the growth and development of DOC going forward. At this time disinfectants used by chicken farmers in general are made from chemical raw materials. All chemical disinfectants used in the field are generally declared safe for humans and animals. But the current trend of human life has begun to shift from chemicals to natural materials. But there is no scientific data to support this information. This study aims to scientifically determine the content of the main active compounds of liquid smoke of rice husk and its potential in antibacterial activity.

Material and Method

This research was conducted in April-November 2019. The process of chemical testing to identify active compounds from liquid smoke of rice husk was carried out at the Organic Chemistry Laboratory, Faculty of Mathematics and Natural Sciences UGM. Antibacterial activity testing was carried out at the Laboratory of Microbiology, Research Center for Biotechnology, Universitas Gadjah Mada.

The equipment was used in this study include ice box, ice pack, tube and stick swab, petri dish, use and spreader, Bunsen, antibiotic blank disc, ruler, incubator, syringe, measuring cup and a set of

GCMS (gas chromatography mass spectrometry) tools. The materials used in this study were liquid smoke of rice husk, aquadest, Mueller Hinton Agar (MHA), Nutrient Agar, alcohol, and neutral saline.

Five liquid smoke of rice husk samples were obtained from Bukata Company is located in Nglipar, Gunungkidul, Yogyakarta, Indonesia. Liquid smoke is sampled from different production batches. Liquid smoke products were analyzed using GCMS (gas chromatography mass spectrometry) to determine the content of active compounds, especially acetate and phenol compounds which are indicated to have the ability as antibacterial. The active fraction of antibacterial liquid smoke of rice husk was analyzed using Gas Chromatograpy-Mass Spectroscopy (GC-MS) Shimadzu QP2010S. A sample of 1 µL was injected into the GC-MS which was operated using a 30 m long glass column, 0.25 mm in diameter and 0.25 µm thickness with a programmable oven temperature between 60-250 °C, Helium carrier gas pressure 13 kPa, a total rate of 40 mL / min and a split ratio of 1:73.

In vitro antibacterial activity testing begins with making a total stock of bacteria. Making a total stock of bacteria is done by collecting broiler chicken coop floor swabs. The litter swab was taken and transported to the Pharmacology laboratory using an icebox and the next process was carried out. The results of the cage floor swabs were planted on the Mueller-Hinton agar medium which has been prepared sterile beforehand. Bacterial planting was carried out by a thorough streak method on the surface of the MHA growing media. Mueller-Hinton agar media which had been planted with bacteria from the cage floor swab was then incubated for 24 hours at 37 ºC (Jain et. al., 2016).

The results of the growing bacteria were stored and cultured in other MHA media as a multiplication of total bacterial stock. The growing bacteria were stored in a nutrient broth solution as stock and stored in a refrigerator at 4ºC. In this case we do not isolate certain bacteria but use all the bacteria that grow from the swab of the broiler litter.

The first step for antibacterial activity was the stock of a disk containing 100% concentrations of liquid smoke. Making the disk is done by dripping 15 µl of the liquid smoke stock suspension solution to the blank disk. The preparation of bacterial solutions to be planted is adjusted to McFarland standards. Intake of bacterial isolates using a sterile loop from total bacterial culture. Bacteria were inserted into the tube and makes the bacteria suspense into normal saline. The tube turbidity was compared it with McFarland standards. If it is not suitable, it can be done by adding bacteria or adding sterile saline and compare with the standard.

Standard bacterial suspense was planted on the MHA using a sterile loop use by streaking evenly on all media surfaces aseptically. The media was left for a while until the bacterial streak dries. After being seen eating dried, a disc containing liquid smoke was planted. In addition to the disk planting method, it is also carried out by the diffusion method by making well in the media and 100 ml of liquid smoke was then added. The disk agar diffusion technique was used to evaluate the antimicrobial ability of liquid smoke. The sterile Mueller-Hinton agar medium was inoculated with standardized test organisms. With the help of a sterile blue tip base with a diameter of 9 mm, a hole was made in the agar medium. As much as 100 µl of liquid smoke was put into the wells, while the central well is filled with the same volume of sterile water for control. The agar media was incubated for 24 hours at 37 °C in an upright position. Inhibition zones were checked for which indicates the degree of sensitivity or antibacterial activity of liquid smoke. The bacterial growth inhibition point is measured from the edge of the well to the outermost point of the inhibition zone. The test was repeated five times for each sample and averaged over all barriers in each case. Antibacterial activity was measured using a ruler and data entered into a database (Jain et. al., 2016).

Chemical active compounds data from the GC-MS analysis were analyzed descriptively to find out the average compound concentration in each liquid smoke sample. Antibacterial activity data were grouped into each group of liquid smoke. Statistical analyses were performed on the basis of each plate as the unit. All data were analyzed statistically using ANOVA analysis to determine differences in each sample group. The differences between groups were estimated with the 99% confidence interval. Statistical analysis data was conducted using SPSS 25.0 version.

Result and Discussion

The results of active compounds of liquid smoke of rice husk were identified using GC-MS, shown in Figure 1. The active compounds of liquid smoke constituents were showed a fragmentation pattern with varying retention times and different area percentages. The pattern of fragmentation and percentage of this area was used as a basis for the analysis of the determination of the main active substances contained in the liquid smoke of rice husks. The main of the active compounds of liquid smoke that have the ability as an antibacterial were identified based on the mass spectrum fragmentation pattern and the percentage of area. The main components of active compounds composing liquid smoke which has the antibacterial ability and the percentage of the largest area can be seen in Table 1. Acetic compounds acid at 25.06%, Cyclopropane, 1,1-dibromo-2-chloro-2-fluoro- at 8.58%, Cyclohexanol, 1-ethynyl-, carbamate at 6.64% and 2-Propanone at 6.07% were the main active ingredient components contained in the liquid smoke of rice husk that has antibacterial ability. The chemical structure of the four major components of liquid smoke has biological activity as an antibacterial. This is consistent with the results of several studies that have been reported by several researchers as summarized in Table 2. These compounds in biological activity have the ability to inhibit the growth of gram-positive (*Staphylococcus aureus, Bacillus subtilis*) and negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*).

GC-MS test results were indicated that several main active compounds in liquid smoke have antibacterial abilities. The results of testing the In Vitro antibacterial activity of liquid smoke were carried out in vitro on the media so that the MHA can be seen in table 3. Based on the results of measurements of bacterial inhibition zones on MHA media showed that the disk method showed fewer tangible results. This is likely due to differences in molecular weight contained in liquid smoke making it difficult to seep from the disk to the media, and the small number of substance that can be accommodated by the disk compared to the amount of substances used in the diffusion method. The diffusion method was showed a real bacterial inhibition zone with an average of 3.62 mm from all test samples. The

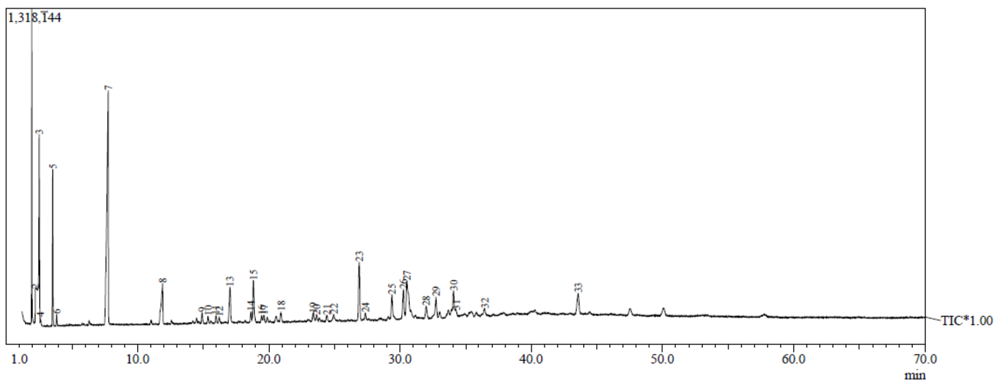

Figure 1. GC-MS chromatogram of active compounds in liquid smoke of rice husk

Table 1. Results of GC-MS analysis of the main chemical components of rice liquid smoke of rice husk that have the ability as an antibacterial

Peak	Time Retention (min)	% Area	Chemical Component	Formula
1	1.941	8.58	Cyclopropane, 1,1-dibromo-2-chloro-2-fluoro-	C3H2Br2ClF
2	2.217	6.64	Cyclohexanol, 1-ethynyl-, carbamate	C9H13NO2
5	3.533	6.07	2-Propanone	C3H6O
7	7.759	25.06	Acetic acid	C2H4O2

Table 2. Biological activities of major active compounds contained in liquid smoke of rice husk

Active Compound	Biological Activity
Cyclopropane, 1,1-dibromo-2-chloro-2-fluoro-	Against Gram-positive (Staphylococcus aureus, Bacillus subtilis) and Gram-negative (Escherichia coli, Pseudomonas aeruginosa) bacteria (Sadyrbekov et al., 2018)
Cyclohexanol, 1-ethynyl-, carbamate	Antibacterial (Dubey et al., 2014)
2-Propanone	Growth inhibitor for Escherichia coli (Kawase et al., 2001)
Acetic acid	Against Gram-negative bacteria including Pseudomonas aeruginosa (Fraise et al., 2013)

Table 3. The average total inhibition zones of bacteria from liquid smoke of rice husk on MHA media

Method	Inhibition zone of each sample (mm)					
	A	B	C	D	E	F-Stat
Disc	0±0	0±0	0±0	1±0	1±0	-
Diffusion	3.10±0.22	2.90±0.96	1.90±0.22	5.20±0.57	5.00±0.61	29.52*

*: P<0.01

ANOVA statistical test results were showed a real difference between the sample groups. Inhibitory zones of liquid smoke of rice husk on MHA media with total inoculation of bacteria originating from broiler chicken coops can be seen in Figure 2. In general, it shows that liquid smoke of rice husk has the antibacterial ability and has the potential to develop be a natural disinfectant.

Figure 2. Antibacterial activity of liquid smoke

Conclusion

Based on the results of the study, it can be concluded that the liquid smoke of rice husk has several main active compounds with the antibacterial ability and has the potential to develop be a natural disinfectant.

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