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Antibacterial Properties of Liquid Smoke from Various Raw Materials with Different Pyrolysis Temperature Level

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Abstract : This study aims to determine the nature of liquid smoke toxicity obtained from pyrolysis on various raw materials with different temperature levels to EscherichiaColi. This study was conducted experimentally by using the complete random design on factorial pattern 3 x 4 x 7 with three replications so that there are 252 experimental units. Factor A is the type of raw material that consists of coconut husk, coconut shell and cinnamon, factors B is temperature levels such as temperature 100 ± 10°C; 200 ± 10°C; 300 ± 10°C; and 400 ± 10°C and factors C is the seven (7) concentration levels of 0 ppm, 1 ppm 10 ppm, 100 ppm, 500 ppm, 1000 ppm and 1500 ppm. The observed parameters are the antibacterial properties of Escherichia coli resistivity diameter (DDH). The result of research showed that there is a significant interaction ($P<0.05$) between using the type of liquid smoke raw material with pyrolysis temperature levels and the difference of liquid smoke concentration to the antibacterial properties such as Escherichia coli resistivity diameter (DDH). The highest value of Escherichia coli resistivity Diameter (DDH) on liquid smoke can be found in raw material treatment from cinnamon with pyrolysis temperature level of 400 ± 10°C at concentration 1500 ppb that shows resistivity diameter (22.29 mm/ppb) compared to the other treatment combinations. Based on this result of research, it can be concluded that the use of cinnamon with pyrolysis temperature level of 400 ± 10°C at concentration 1500 ppb is better to be used as antibacterial than the combination of coconut husk and coconut shell raw materials treatment, the other pyrolysis temperature and lower liquid smoke concentration.
Keywords : raw material type; temperature; liquid smoke; concentration; antibacterial properties.

I. Introduction

The antibacterial substance is the compound that can kill or inhibit the growth of microorganisms. It is the substance that can inhibit (bacteriostatic) or kill the microbes (bactericidal), particularly harmful microbes for humans [1]. Microbes are microscopic organisms which among others consist of bacteria, fungi, and virus [2]. In its interaction with humans, some of those microbes can be harmful. For examples, pathogenic bacteria Escherichia coli and group of bacteria Coliform can cause gastrointestinal disease [2]

One of the efforts to fight those microbes is by using liquid smoke that has antagonist properties (antimicrobial) as an intrusion or inhibit on other microbes metabolism. Liquid smoke that has antimicrobial capabilities can produce antimicrobial compounds. Antimicrobial compounds that are produced by liquid smoke such as phenols, carbonyls are compounds that can inhibit the growth of bacteria. For self-defense and

competition with other microbes in getting nutrition, habitat, oxygen, light and others. Those antimicrobial compounds can be classified as antibacterial or antifungal [3].

Based on the research results[4] that the liquid smoke from coconut shell cam inhibit the growth of Escherichia coli and Staphylococcus aureus microbes. Wood vinegar, acacia and rubber for food products as preservative for milkfish, globefish and catfish with a concentration of 10% can preserve fish for two months [5]. According to[40] that the result of this research, it can be concluded that using cinnamon with level pyrolysis temperature 400±10oC is better used rather than coconut fibre and coconut shell.

Escherichia coli bacteria is one of the main species from negative gram bacteria. Generally, it can cause various diseases when it enters into the organs or other tissues. Escherichia coli bacteria can cause pneumonia, endocarditis, wounds infection and abscesses in various organs. This rod-shaped bacteria is the main organisms in the colon, lives as commensalism in the human body and it has a role in the formation of vitamin K that is important for blood clotting [6].

All types of wood distillate contain compounds that can be extracted as phenol derivatives, which can inhibit the growth of microbes. Liquid smoke from wood is used as preservative because of the similarity of wood distillate chemical components that can be found in certain types of preservatives, where the components that act as preservatives are phenol and its derivatives. The efforts in providing added value from plantations waste crop that are still yet to receive optimal treatment such as coconut husk, coconut shell and cinnamon in West Sumatra need to be further experimented on liquid smoke antibacterial properties. The purpose of this research is to determine the antibacterial properties of liquid smoke from treatment combination of raw materials such as coconut husk, coconut shell and cinnamon with pyrolysis temperature and different liquid smoke concentration to Escherichia coli bacterium.

II. Materials and Methods

Tools and instruments used in this study include laboratory glassware, test tube rack, aluminium foil, evaporator filter paper, vortex, desiccator, hot plate, aerator, oven, analytical scales, blenders, label paper, rulers, pencils, aluminium foil, plastic, filter paper, cotton, Erlenmeyer flask, Becker glass, measuring cups, funnels, test tubes, rod stirrer, pipette, glass bottles, weighing bottle, measuring cups, oven, glassware commonly used in the microbiology laboratory, a set of rotary vacuum evaporator, volume pipettes, micro pipettes, tweezers, perforator, autoclaves, and scales, as well as one (1) set of laboratory-scale liquid smoke tools maker [7].

Materials and chemical reagents in this study are coconut husks and coconut shells waste from Padang central market and cinnamon that its outer skin has already been taken is obtained from the cinnamon farmers in Tanah Datar. Distilled water, methanol, Nutrient Jelly (NA), Nutrient Broth (NB), Escherichia coli bacteria ATCC 11778, KCl, milk, sugar solution and NaCl.

*2.1.*Research Implementation

The stages in this research implementation consist of three phases:

2,1,1, Liquid smoke pyrolysis tool assembling

The circuit of liquid smoke extraction tool is made at laboratory scale, it is based on the research result and characteristics of liquid smoke[7]. In this study, it used liquid smoke tool maker that consists one unit of complete condenser equipment with water storage in the form of drum at capacity 100 liters equipped with water pump to circulate the cooling water with 14 meter hose for water circulation, a container for liquid smoke in form of 5 Erlenmeyer tube with capacity of 500 ml, stainless steel kiln with capacity of 3 kg and LPG stove burner that at the end of the pipe is equipped with vacuum pump to draw the burning smoke in order to obtain liquid smoke as shown in Figure 1 below:

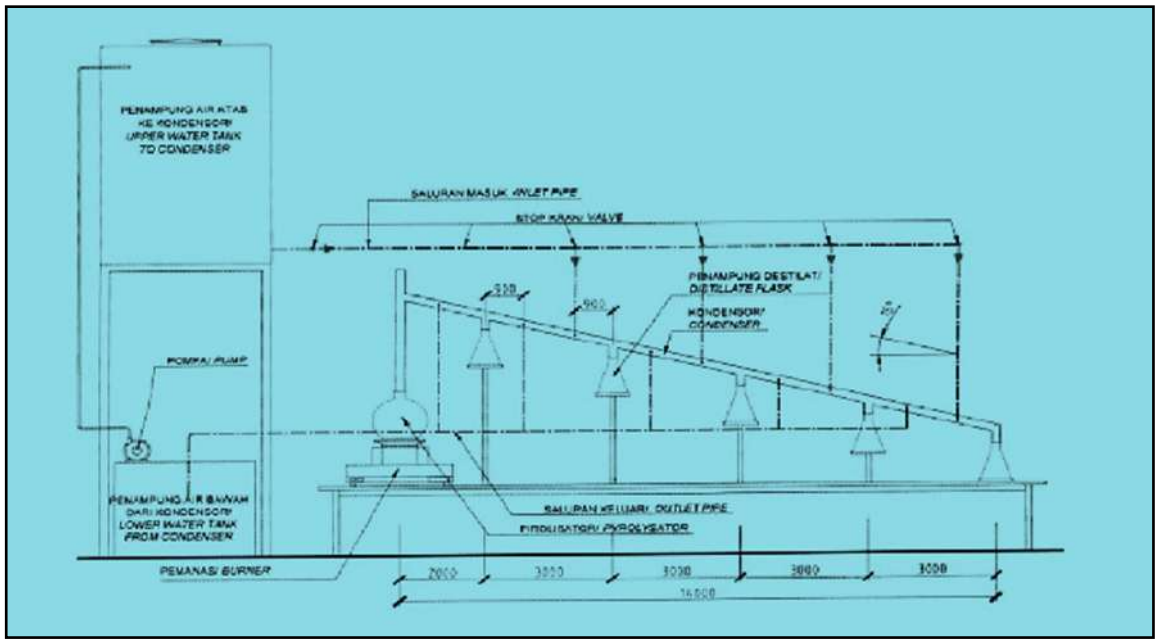

Fig. 1 Laboratory scale liquid smoke tool maker

*2.1.2.*The pyrolysis process (making) of liquid smoke

Research on making liquid smoke by using pyrolysis refers to the above research activities as an input to redesign the laboratory scale liquid smoke tool maker. After this liquid smoke tool maker has been assembled, then it is continued with producing liquid smoke. This process begins from raw material preparation by providing coconut fiber, coconut shell, and dry cinnamon, the weight of each raw material is about 40 kg with moisture content ranging from 4-10%, cleaned from dirt. The next raw materials are cut into the smaller size with similar size ± 4-9 cm2. Then, further activity puts the raw materials into pyrolysis reactor for five (5) hours, the weight of each sample is 3 kg at the temperature of 100 ± 10°C; 200 ± 10°C; 300 ± 10°C; 400 ± 10°C, using LPG fuel burner stove. The pump is used to drain water from the water source to the condenser. Burner and water pump are switched on simultaneously. Distillate container (liquid smoke) is placed inside glass bottles. The temperature is measured by using thermometer and measurements are performed every ½ hour that is measured at several places in pyrolysator, distillates container, water resources, as well as the inlet and outlet of the condenser. After 5 hours, it will obtain three fractions, which are the solid fraction in form of carbon, heavy fraction in form of tar and light fraction in form of smoke and gas methane. Then, light fraction is passed into condensation pipe in order to obtain liquid smoke while methane gas remained gas and is not condensed. The obtained liquid smoke is further precipitated for 1 (one) week, after that the analysis is performed. The purpose of precipitation for 1 (one) week is to precipitate dirts that exist in liquid smoke. After 1 (one) week liquid smoke is precipitated, then the antibacterial test is performed.

2.1.3.Antibacterial test by using Kirby-Bauer disc method

This test [8], [9] includes the following stages:

1. *Sterilization Equipment and Materials*: Sterilization is implemented in an appropriate manner for each tool. The tools that will be sterilized are washed and dried beforehand. Test tubes, beakers, Erlenmeyer is covered its mouth with cotton. Furthermore, it is sterilized in an autoclave at the temperature of 121^{o}C for 15 minutes. Tweezers, needle are sterilized with flumber at Bunsen flame. The microbiological test is performed aseptically in laminar air flow (LAF) that is previously sterilized with UV light and is sprayed with 70% alcohol. The sterilization is performed 2 hours before work and after work therein.
2. *Making of Growth Media:* - Nutrient jelly (NA), weight at 23 grams NA (nutrient jelly), dilute with 1 litre of distilled water, heat until everything is dissolved, and sterilize in an autoclave at 121°C for 15 minutes at 1 atm pressure [10]. The composition of nutrient jelly (g/l): meat extracts 1%, peptone 1%, and jelly 1.5% [11]. - Nutrient Broth (NB), weigh 8 grams of NB, dilute with 1 liter of distilled water, heat until everything is dissolved, insert into Erlenmeyer, and sterilize in the autoclave at 121°C for 15 minutes at 1

atm pressure [12]. The composition of nutrient broth (g/l): lab lemco powder 1%, yeast extract 2%, peptone 5% and 5% NaCl.

3. *Making Test Solution:* In determining the highest activity of liquid smoke as the result of raw materials treatment combination (coconut fiber, coconut shell and cinnamon) with different pyrolysis temperature (temperature of 100 ± 10°C; 200 ± 10°C; 300 ± 10°C; and 400 ± 10°C) at different liquid smoke concentration (0 ppm, 1 ppm 10 ppm, 100 ppm, 500 ppm, 1000 ppm and 1500 ppm).
4. *Breeding of Test Bacteria*: Test bacteria is inoculated into 5 ml tilted nutrient jelly by using sterile needle through scraping Escherichia coli bacteria. ATCC 11778 at the end of needle to tilted nutrient jelly media, then it is incubated at 37°C for 18-24 hours.
5. *Preparation of Bacterial Suspension:*Bacteria from the pure breeding result from tilted nutrient jelly (NA) that after being inoculated it reach the age of 18-24 hours. At 37°C, it is inoculated at 1 oze in 10 ml. Nutrient Broth (NB) then is incubated at 37°C for 18-24 hours. After that, the turbidity is synchronized with 0.5 Me solution. Farland or equal with the number of bacteria 1 x 108 CFU/ml (CFU: Colony Forming Unit) or 250-300 colonies on solid media. Next, in order to obtain the bacterial suspension containing 106 CFU/ml, is by taking 1 ml (from the tube containing 108 CFU/ml) to be mixed with 9 ml NaCl 0.9% sterile. Then, we will get bacterial suspension with a density of 107 CFU/ml. Followed by taking 1 ml again (from the tube containing 107 CFU/ml) to be mixed with 9 ml of natrium broth in order to obtain the suspension with the density of 106 CFU/ml [13]-[15].
6. *Bacteria Identification with Gram Coloring:* A total of one oze of bacteria in nutrient jelly is fixated on a clean microscope slide. Bacteria spread is added with violet gentian of excess state, then we wait for one minute. The excess of dye is disposed, then the slide is rinsed with running water. The growth media is dried over methylated spirit fire. After drying, the excess of lugol solution is added to that growth media surface and wait for 1 minute. After 1 minute, the growth media is rinsed with running water. The growth media is rinsed with 90% alcohol until all the color dye is faded and then it is washed with running water. The growth media is dried over methylated spirit flame. After drying, the excess of safranin is added to the growth media surface and wait for 45 seconds. The growth media is washed with water and dried. The growth media is then added with 1 drop of immersion oil and observed by using Olympus CX21 microscope with 100 times magnification [16], [17].
7. *Antibacterial Activity Test by Disc Diffusion Method*: Antibacterial activity test is implemented by using disc methods. Sterile Cylinder disc that is used has the diameter of 7 mm. Sterile NA liquid media is poured aseptically 20 ml in 9 cm diameter petri dish until it is even, then wait until it is freezing. Next, bacteria suspension E Coli that its turbidity has been standardized is dipped with the sterile cotton stick, wait shortly so that the liquid seeps into the cotton. Then the stick is lifted and squeezed by pressing the stick on the inner tube wall while rotating it around the tube. The cotton sticks are scratched on the surface of NA media until the entire media surface is covered with scratches. NA Media is left for 5-15 minutes so that suspense bacteria seeps into the jelly. Then, the solution of 100 ml liquid smoke is used with a concentration of 0 ppm, 1 ppm, 10 ppm, 100 ppm, 500 ppm, 1000 ppm and 1500 ppm is dropped on the cylinder disc. It is incubated at 37°C for 18-24 hours. After it is incubated, the antibacterial force that occurs is determined by measuring the diameter of growth resistivity area (DDH) by using callipers [13], [18], [19].

2.2.Experimental Design

The study is conducted using a complete random design (RAL) factorial 3 x 4 x 7 with 3 replication in order to obtain 252 experimental units. Factor A is the type of raw material that consists of coconut husk, coconut shell and cinnamon, factor B is the pyrolysis temperature level of 100 ± 10°C; 200 ± 10°C; 300 ± 10°C; and 400 ± 10°C and factor C is liquid smoke concentration level of 0 ppb, 1 ppb, 10 ppb, 100 ppb, 500 ppb, 1000 ppb and 1500 ppb. The observed parameters are the measurement of diameter formed by the treatment given. Furthermore, the data are analyzed by variant investigation analysts 5%, if there is a significant difference it will be followed by Tukey's test 5% [20].

III. Results and Discussions

3.1.The Effect of Different Raw Materials Treatment Combination with Pyrolysis Temperature against DDH Escherichia coli

The results of variant investigation analysis shows that there is interaction on the different liquid smoke raw material treatment combination with different pyrolysis temperature to the value of liquid smoke antimicrobial ($P<0.05$), the treatment combination of different pyrolysis temperature with concentrations as well as different raw materials combination, the pyrolysis temperature, and different liquid smoke concentration also shows the interaction or provides significant effect.

The chosen method in liquid smoke antibacterial activity test from different raw materials at different pyrolysis temperatures is jelly diffusion method. The reason for choosing this method because it is quick, easy and simple in process. In the determination of antibacterial activity, the formation of resistivity zone (clear zone) around the paper disc after being dipped beforehand to the test solution proves that terbut compound has antibacterial activity. Resistivity zone diameter is an indication of testing bacterial sensitivity, the greater resistivity zone then that antibacterial has better antibacterial activity. The result of variant investigation analysis on antimicrobial properties (resistivity diameter) of liquid smoke to Escherichia coli bacteria.

Observations on average resistivity diameter (DDH) to microbial Escherichia coli on different raw materials treatment combination with pyrolysis temperature is presented in Table 1.

Table 1.The Result of Average DDH (mm/ppb) Escherichia coli Observations, Based on Different Raw Materials Treatment with Pyrolysis Temperature.

Factor and level	Pyrolysis temperature (oC) (T)				Average (T)	Interaction (T x B)
	100 (T_1)	200(T_2)	300 (T_3)	400(T_4)		
Coconut fiber (B_1)	2.82±0.23 ^{a}	7.93± 1.08 ^{abc}	10.37 ± 1.1 ^{ab}	5.25 ± 1.23 ^{bc}	6.59± 1.13 ^{a}	-0.45
Coconut shell (B_2)	5.94 ± 1.03 ^{bc}	8.24 ± 1.22 ^{abc}	9.38 ± 1.12 ^{abc}	3.06 ± 0.91 ^{a}	6.66± 1.13 ^{a}	2.02
Cinnamon (B_3)	7.16 ± 1.24 ^{bc}	14.18 ± 0.55 ^{c}	10.20 ± 1.18 ^{ab}	6.95 ± 1.29 ^{bc}	9.62± 1.20 ^{b}	2.03
Average of raw materials (B)	5.31± 1.02 ^{a}	10.12± 1.09 ^{c}	9.99± 1.03 ^{b}	5.09± 0.91 ^{b}	5.31	
Interaction (B xT)	-0.81	-3.96	-0.55	-2.59		

Notes: Different superscript letters in average columns shows significant difference ($P<0.05$)

Based on Table 1 shows the positive interaction value on the raw material type treatment combination of coconut shell and cinnamon with different pyrolysis temperature (line), while the treatment combination of coconut fiber raw material with different pyrolysis temperature has negative interaction value. Further, on treatment combination of pyrolysis temperature with different types of raw materials (columns) indicates the negative interaction value. Positive interaction value means that the influence from both factors of raw materials and concentration of liquid smoke gives respond simultaneously to the increase of resistivity diameter value E. coli, compared to the treatment of each factor alone. Then, the negative interaction value means that both factors give the opposite response or responses given by those two factors on resistivity diameter value on Escherichia coli is lower than the performance on each factor alone.

Table 1 shows the resistivity diameter of Escherichia Coli bacteria is greater on the interaction of different raw materials treatment with pyrolysis temperature contained in cinnamon raw material with pyrolysis temperature of 200±10°C of 14.176 mm/ppb is not significantly different with cinnamon treatment combinations on pyrolysis temperature of 300±10oC and has significant different with other treatment combination, while the smallest resistivity diameter can be found in coconut fiber raw material at pyrolysis temperature of 100±10°C by 2.82 mm/ppb. The size of resistivity diameter on cinnamon at 200^{o}C is because of many components in cinnamon that it has strong antimicrobial ability. the criteria of the antibacterial strength is as follows [21]: resistivity zone diameter of 5 mm or less is categorized as weak, resistivity zone of 5-10 mm is average, resistivity zone of 10-20 mm is considered strong and resistivity zone 20 mm or more is categorized

very strong. Based on these criteria, then the antibacterial power of liquid smoke on Escherichia coli bacteria in the cinnamon raw material with pyrolysis temperature of 200±10°C of 14.176 mm/ppb is relatively strong.

The regression equation shows the average DDH Escherichia coli in cinnamon liquid smoke is Y = -0,535x + 10.9; r2 = 0.0401, coconut shell Y = 0,973x + 4.16; r2 = 0.1475, coconut fiber Y = -0,821x +8.77; r2 = 0.1457 at four different pyrolysis temperatures. The three regression equation shows the relationship between the type of raw material with pyrolysis temperature is very weak with R2 respectively coconut fiber 0.1457, coconut shells 0.1475 and coconut fiber 0.0401. This illustrates that liquid smoke raw material type with different pyrolysis temperature does not really correlate to resistivity diameter of Escherichia Coli bacteria. It is caused by the contents of phenol and carbonyl in the liquid smoke is still not strong enough in the process of resistivity against microbes. The quantity of phenol in wood liquid smoke varies greatly, which is between 10-200 mg/kg [22]. Some types of phenol that are commonly found in the smoked product are guaiacol and siringol. This phenol compounds that can inhibit the growth of bacteria that it can form resistivity zone diameter.

The greater concentration of liquid smoke that is added, then the greater resistivity zone diameter that is formed. This is because the liquid smoke contains compounds of phenol, carbonyl, aldehyde and acetic acid in liquid smoke that acts as antibacterial thus affecting the growth of testing bacteria as indicated by the formation of a clear zone. The working mechanism of antibacterial compounds in liquid smoke, such as phenols, aldehydes and acetic acid compounds on inhibiting the growth of both test bacterial is through denaturalization of enzymes and damaging the cell membrane of test bacteria, breaking the hydrogen bonds. The structure of the cell wall can be damaged by inhibiting its development or change it after being formed [23].

3.2.The Effect of Different Raw Materials Treatment Combination with Liquid Smoke Concentration against Insipid Force Diameter (DDH) Escherichia coli

The results of variant investigation analysis shows that there is interaction on different treatment combination in raw materials with different concentrations of liquid smoke. Average observations resistivity diameter (DDH) of microbial Escherichia Coli on different raw materials treatment combination with liquid smoke concentration is presented in Table 2.

Table 2.Average Observations of DDH (mm/ppb) Escherichia coli Based on Treatment of Different Raw Materials with Liquid Smoke Concentration

Factor	Liquid Smoke Interaction (K)							Average (K)	Interaction
And level	0 ppm (K_0)	1 ppm (K_1)	10 ppm (K_2)	100 ppm (K_3)	500 ppm (K_4)	1000 ppm (K_5)	1500 ppm (K_6)		B*K
Coconut fiber (B_1)	0.83±0.04	0.87±0.05	3.80± 1.31	6.04±1.43	6.87±1.2	9.97± 1.48	17.75±2.39	2.89±0.97	-6.82
Coconut shell (B_2)	0.83±0.04	0.91±0.05	3.21±1.15	5.69±1.98	8.46±1.6	8.92± 1.84	18.58±1.23	2.66±1.26	-7.10
Cinnamon (B_3)	3.29±1.37	3.49±1.69	6.45± 1.89	6.65±1.05	12.66±1.74	13.6± 1.83	20.50±2.1	4.97±1.03	-7.43
Average(B)	1.65±1.88	1.76±1.82	4.49± 1.49	6.13±1.35	9.34±1.79	10.83± 1.04	18.95± 1.56	3.51	
Interaction (K*B)	-1.64	-1.72	-2.16	-0.64	-2.80	-3.13	-1.274		

Notes: Different superscript letters in average columns shows significant difference (P<0.05)

Table 2 shows the negative interaction value both on a treatment combination of different types of raw materials with different concentrations of liquid smoke (rows) and on the (column) combination of liquid smoke concentration treatments with different types of raw materials. If there is negative interaction value, it means both factors give the opposite response or responses given by those two factors on resistivity diameter value (DDH) Escherichia coli is lower than the performance of each factor alone.

Table 1 shows resistivity diameter of Escherichia Coli bacteria is the biggest on the treatment combination of different raw materials with pyrolysis temperature contained in cinnamon raw material with the

concentration of liquid smoke 1500 ppb of 20,500 mm/ppb is not significantly different with cinnamon treatment combinations on concentration of 1000 ppb and it is significantly different with the combination of other treatments, while the smallest resistivity diameter can be found in coconut fiber raw materials at liquid smoke concentration of 0 ppb. The size of resistivity diameter in cinnamon at 1000 ppm is because there are many components in cinnamon that it has the ability to be a powerful antimicrobial. On coconut fiber raw material with the concentration of 0 ppm (no liquid smoke) the resistivity diameter value is low because it cannot inhibit bacterial growth of Escherichia coli, most likely because of the composition of Gram-negative bacteria cell wall is too complex. Most of Gram-negative bacteria have complex lipopolysaccharide on the cell wall. Those substances are endotoxins, the structure of endotoxin lipopolysaccharide in the cell wall, namely, polysaccharide-O-specific which is the somatic antigen of thin colonies that induce specific immunity, general polysaccharide core (rough colony antigen) that induces some unspecified resistance to sepsis Gram-negative, lipid A with KDO (acid-2-keto-3-deoxy-octanoate), which is responsible for the primary poisoning [1].

The result shows that liquid smoke from raw materials with different pyrolysis temperature has the ability to inhibit the growth of Escherichia Coli bacteria. This can be seen from the clear zone that is formed around the disc paper dipped in liquid smoke. The higher smoke concentration, then the resistivity zone become bigger. Many factors cause the resistivity zone to be not evenly rounded, one factor is the media seeding in Petri dish, the growing bacteria population is not concentrated in one part while the other section of the population is very concentrated. This is because at the time of making seeding layer of bacteria, the bacterial spread is not even. Consequently, discs that contain antibacterial substances that are placed on the bacteria population is not concentrated will form a larger resistivity zone compared to disc paper that is placed at the concentrated place of bacteria population.

Furthermore, the criteria of antibacterial strength are as follows [21]: resistivity zone diameter of 5 mm or less categorized as weak, resistivity zone of 5-10 mm is average, resistivity zone of 10-20 mm is considered strong and resistivity zone of 20 mm or more is categorized as very strong. Based on these criteria, the antibacterial power of liquid smoke on Escherichia coli bacteria from the cinnamon raw material with a concentration of 1000 ppm is very strong.

The regression equation on coconut fiber raw material $y = 2.6623x-3.9892$; $r^2 = 0.8609$, $y = 2.5732x$ coconut shell-3.7009; $r^2 = 0.8827$ and $y = 2.7873x$ cinnamon - 1,626; $r^2 = 0.9072$ at different concentrations of liquid smoke. Based on regression equation, it shows the relationship between the type of raw material with the concentration of liquid smoke has a strong relationship to resistivity diameter (DDH) of Escherichia Coli with R2 respectively 0.8609, 0.8827 and 0.9072. The relation of cinnamon raw material with different concentrations of liquid smoke shows the strongest correlation of 0.9072, it means that 90.70% resistivity diameter (DDH) of Escherichia Coli is influenced by relationship on raw material with the concentration of liquid smoke, while the rest is influenced by the other factors. The strength of relationship on cinnamon raw materials with different concentrations is because there are many components that can be found in cinnamon liquid smoke that showing the strong correlation to antibacterial properties.

3.3.The Effect of Different Pyrolysis Temperature Treatment Combination with Liquid Smoke Concentration against DDH Escherichia Coli

The results of variant investigation analysis shows that there is interaction on a combination treatment of different pyrolysis temperature with different concentrations of liquid smoke ($P<0.05$). Average observation on resistivity diameter (DDH) of Escherichia Coli on Escherichia Coli microbes in the treatment combination of the different pyrolysis temperature treatment with liquid smoke concentration can be presented in Table 3 below:

Table 3.Average Observation on DDH (mm/ppb) of Escherichia Coli Based on Different Pyrolysis Temperature Treatment with Liquid Smoke Concentration.

Factor	Pyrolysis temperature (°C) (T)				Average (T)	Interaction
And level	100 (T_1)	200(T_2)	300 (T_3)	400(T_4)		(T*K)
0 ppm (K_0)	0.7974±0.04 ^{g}	4.0674±1.86 ^{fg}	0.8531±0.02 ^{g}	0.8821±0.01 ^{g}	1.65± 1.88 ^{e}	0.659
1 ppm (K_1)	0.8369±0.02 ^{g}	4.3612±1.18 ^{fg}	0.9445±0.02 ^{g}	0.8937±0.02 ^{g}	1.761.49 ^{de}	0.715
10 ppm (K_2)	0.8917±0.06 ^{g}	5.1453±1,3 ^{fg}	11.018±1.2 ^{cde}	0.9007±0.02 ^{g}	4.491.49 ^{de}	-0.202
100 ppm(K_3)	0.9195±0.08 ^{g}	11.596±1.65 ^{cde}	11.08±0.85 ^{cde}	0.9278±0.03 ^{g}	6.13± 1.79 ^{c}	0.946
500 ppm(K_4)	8.7409±6.07 ^{def}	12.879±0.98 ^{cde}	11.654±1.12 ^{cde}	4.0677±1.69 ^{fg}	9.34± 1.79 ^{c}	3.511
1000 ppm(K_5)	9.1828±1.31 ^{def}	13.074±1.55 ^{cd}	13.219±1.12 ^{cd}	7.854±1.26 ^{ef}	10.83± 1.04 ^{b}	2.096
1500 ppm(K_6)	15.785±1.87 bc	19.69±1.44 ^{ab}	21.151±1.02 ^{a}	19.491±1.71 ^{ab}	19.03± 1.56 ^{a}	0.922
Average (K)	5.31± 1.02 ^{a}	10.12± 1.09 ^{a}	9.99± 1.03 ^{b}	5.00± 0.91 ^{b}	7.60	
Interaction (K*T)	6.62	6.14	7.68	6.94	6.84	

Notes: Different superscript letters in average columns shows significant difference (P<0.05)

Table 3 shows the positive interaction value in the treatment combination of liquid smoke concentration on 0 ppm. 1 ppm, 100 ppm, 500 ppm, 1000 ppm and 1500 ppm (rows) with different pyrolysis temperature, then the treatment combination of liquid smoke concentration of 10 ppm shows negative interaction. In the treatment (column) of different pyrolysis temperature with liquid smoke concentration shows positive interaction. If the interaction value is negative, it means that both factors provide an opposite response or the responses given by those two factors on resistivity diameter value (DDH) of Escherichia Coli is lower than the performance on each factor alone, while the positive interaction value means that both treatment factors simultaneously give response in increasing Resistivity Diameter value (DDH) of Escerichia Coli.

Table 3 also shows that the biggest resistivity diameter of Escherichia Coli bacteria in the treatment combination of liquid smoke concentration with pyrolysis temperature can be found in the concentrations of liquid smoke on 1500 ppb at pyrolysis temperature of 300±10°C at 21.151 mm/ppb is not significantly different with the combination of liquid smoke concentration on 1500 ppb at pyrolysis temperature of 200±10°C, 400±10°C and it is significantly different with the combination of other treatments, while the smallest resistivity diameter can be found in the liquid smoke concentration of 0 ppm (no liquid smoke) at pyrolysis temperature of 100±10°C of 0.797 ml/ppb. The size of the resistivity diameter at concentrations of 1500 ppb is because there are many components contained in the liquid smoke that it has the ability to be a powerful antimicrobial. The antimicrobial activity of liquid smoke is caused by phenol and acid components. Liquid smoke contains acid and its derivatives (formate, acetate, butyrate, propionate, and methyl ester), alcohol (methyl, ethyl, propyl, alkyl, and isobutyl alcohol), aldehydes (formaldehyde, acetaldehyde, furfural and methyl furfural), hydrocarbons (Silene, cumene, and cymene), ketones (acetone, methyl ethyl ketone, methyl propyl ketone, and ethyl propyl ketone), phenol, pyridine, and methyl pyridine [24]. Phenols and acids are compounds that act as antimicrobial [25]-[32].

The antimicrobial activity mechanism of phenol and its derivatives include reaction with cell membranes that causes increased permeability of cell membranes and results in loss of cell contents, inactivation of essential enzymes and the destruction or inactivation of functional genetic material [36]. The higher concentration of phenol, it will further precipitate all proteins cells, conversely, the lower concentration will further effectively inhibit the enzymes essential.

The regression equation at pyrolysis temperature of 100oC 2.6041x y = -5.4139; r^2 = 0.6634, pyrolysis temperature of 200oC y = 2.4823x - 4.6214; r^2 = 0.8, pyrolysis temperature of 300oC y = 2.5724x - 0.1735; r^2 = 0.9054; and pyrolysis temperature of 400oC y = 3.0742x - 2.3084; r^2 = 0.8667 at different concentrations of liquid smoke. The regression equation indicates that the relationship between pyrolysis temperature with liquid smoke concentrations has a rather strong to a strong relationship to the resistivity diameter (DDH) of Escherichia Coli with R^2 respectively 0.6634, 0.8, 0.9054 and 0.8667. The relationship of pyrolysis temperature at 200±10°C with different concentrations of liquid smoke shows the strongest correlation at 0.9054 means that 90.54 percent of resistivity diameter (DDH) Escherichia Coli is influenced by the relationship of pyrolysis temperature with the concentration of liquid smoke, while the rest is influenced by other factors. The strength of

relationship between pyrolysis temperature with different concentrations liquid smoke is because there are lots of chemical components that can be extracted in pyrolysis time so that it shows a quite strong relationship to its antibacterial properties.

3.4.The Effect of Different Treatment Combination in Raw Materials, Pyrolysis Temperature and Concentration of Liquid Smoke to DDH Escherichia Coli

The results of variant investigation analysis show that there is interaction in the different treatment combination of raw materials, pyrolysis temperature and the different concentration of liquid smoke also shows the interaction or give a significant effect (P<0.05). Average observation on resistivity diameter (DDH) in Escherichia coli microbe in different treatment combination of raw material, pyrolysis temperature with liquid smoke concentrations is presented in Table 4.

Table 4.Average Observation on DDH (mm/ppb) of Escherichia Coli Based on the Treatment of Raw Materials, Pyrolysis Temperature with Liquid Smoke Concentration

Factor And level	Pyrolysis Temperature (oC) (T)	Liquid smoke concentration (ppm) (K) 0 (K_0)	1 (K_1)	10 (K_2)	100 (K_3)	500 (K_4)	1000 (K_5)	1500 (K_6)	Average Temperature /Material (T*B)	Interaction (T*B)
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
Coconut fiber (B_1)	100(T_1)	0.77± 0.02	0.80± 0.04	0.82± 0.02	0.81± 0.03	0.9~3± 0.03	0.99± 0.0002	14.7± 0.03	2.82± 1.96	-3.53
	200(T_2)	0.81± 0.04	0.88± 0.02	0.9± 0.01	10.26± 0.02	12.78± 0.02	13.45± 0.02	16.4± 0.03	7.93± 1.5	-8.21
	300(T_3)	0.84± 0.03	0.94± 0.03	12.6± 0.05	12.12± 0.05	12.83± 0.03	13.43± 0.03	19.823± 0.03	10.4± 1.63	-7.86
	400(T_4)	0.89± 0.05	0.87± 0.03	0.88± 0.03	0.963± 0.002	0.99± 0.03	12.08± 0.03	20.067± 0.03	5.2± 1.35	-7.06
Average (B_1)		0.82± 0.04	0.87± 0.05	3.80± 5.31	6.04± 1.43	6.88± 6.2	9.98± 5.48	17.7± 2.39	6.592	0.29
Interaction (B1*T)		-0.063	-0.042	-1.984	-0.387	-0.051	-5.543	-3.236	-1.62	
Coconut shell (B_2)	100(T_1)	0.76± 0.02	0.84± 0.01	0.93± 0.02	0.98± 0.00	11.79± 0.03	11.81± 0.03	15.36± 2.883	5.9 ± 1.2	-7.48
	200(T_2)	0.85± 0.02	0.93± 0.03	0.94± 0.02	10.75± 0.003	10.27± 0.06	11.13± 0.03	21.3± 0.05	8.24 ±1.34	-8.56
	300(T_3)	0.84± 0.03	0.97± 0.03	10.09± 0.02	10.17± 0.03	0.89± 0.003	11.83± 0.05	21.58± 0.03	9.38± 1.71	-6.44
	400(T_4)	0.86± 0.03	0.89± 0.01	0.89± 0.03	0.88± 0.03	11.81± 0.03	0.89± 0.002	16.12± 1.2	3.06± 1.46 c	-5.45
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
Average (B_2)		0.83± 0.04	0.91± 0.05	3.21± 1.15	5.69± 1.98	8.46± 1.6	8.91± 1.84	18.58± 1.23	6.657	0.75
Interaction (B_2*T)		-0.05	-0.03	-1.51	1.84	-0.01	5.34	-0.42	0.737	
Cinnamon (B_3)	100(T_1)	0.86± 0.01	0.86± 0.02	0.93± 0.03	0.97± 0.01	14.41± 0.05	14.79± 0.03	17.32± 0.005	7.16± 7.46	-8.93
	200(T_2)	10.55 ±0.05	11.26 ±0.01	13.59± 0.02	13.77± 0.02	14.06± 0.05	14.6± 0.003	21.34± 0.003	14.17± 1.32	-3.60
	300(T_3)	0.88± 0.02	0.92± 0.02	10.35± 0.05	10.95± 0.05	11.867 ± 0.03	14.4±0.02	22.05± 0.003	10.2± 1.08	-8.69
	400(T_4)	0.89± 0.03	0.93± 0.03	0.92± 0.01	0.94± 0.0003	10.32± 0.05	10.58± 0.03	22.29± 0.005	6.9± 1.79	-8.69
Average (B3)		3.29± 4.37	3.49± 4.69	6.45± 5.89	6.66± 6.05	12.66± 1.74	13.6± 1.83	20.5± 2.1	9.624	0.46
Interaction (B_3*T)		-0.02	1.70	0.55	0.49	2.41	2.15	-2.60	0.67	
Average of Pyrolysis temperature (T)	100(T_1)	0.79± 0.04	0.84± 0.02	0.89± 0.06	0.92± 0.08	8.74± 6.07	9.18± 1.31	15.78± 1.87	5.308	-6.68
	200(T_2)	4.07± 1.86	1.36± 5.18	5.14± 1.3	11.59± 1.65	12.87± 0.98	13.07± 1.55	19.69± 1.44	10.116	-6.86
	300(T_3)	0.85± 0.02	0.94± 0.02	11.02± 1.2	11.08± 0.85	11.65± 1.12	13.2± 1.12	21.15± 1.02	9.989	-8.13
	400(T_4)	0.88± 0.01	0.89± 0.02	0.9± 0.02	0.92± 0.03	4.07± 4.69	7.8± 5.26	19.49± 1.71	5.002	-6.52
Interaction (T)		0.49	0.54	-0.98	0.08	2.54	0.64	-2.10		
Average concentration (K)		1.65	1.76	4.49	6.13	9.34	10.83	18.95	7.592	
Interaction (B*T*K)		-1.24	-1.31	-1.32	-0.31	-2.89	-1.81	-1.38		

Notes: Different superscript letters in average columns shows significant difference (P<0.05)

Table 4 shows the value of a negative interaction on the different treatment combination in raw materials, pyrolysis temperature and differences liquid smoke concentration to resistivity diameter of Escherichia Coli bacteria. The results of negative interaction mean the three treatment factors simultaneously provide the response to the Resistivity Diameter (DDH) of Escherichia Coli, but the opposite direction, or in other words, the response given from these three factors simultaneously against DDH Escherichia Coli is weaker than the response performance from each factor alone. The results of Resistivity Diameter (DDH) of Escherichia Coli in treatment combination of liquid smoke concentrations with pyrolysis temperature at different concentrations can be found in cinnamon raw material at pyrolysis temperature of 400±10°C in liquid smoke concentration 1500 ppb of 22.29 ppb is not significantly different with liquid smoke made from cinnamon at pyrolysis temperature of 200±10°C and 300±10°C and it is significantly different with the combination of other treatments, while the smallest resistivity diameter is contained in the coconut shell liquid smoke at pyrolysis temperature of 100±10°C in concentrations of liquid smoke 0 ppb (without liquid smoke) at 0.764 ml/ppb. The size of resistivity diameter in cinnamon liquid smoke on pyrolysis temperature of 400±10°C at the concentration of 1500 ppb is because there are many components contained in the liquid smoke that has the ability to be a powerful antimicrobial.

Table 4 also shows that the antibacterial activity will decrease along with the decrease concentration of liquid smoke, but at the lowest concentration of 10 ppb, liquid smoke still indicates its activity. The solubility in the liquid smoke is strong [35], so that the liquid smoke can still diffuse into experiment media of the place to growth the bacteria and chemical compounds on wood vinegar, one of them is phenol, so that resistivity zone is larger. Phenols contained in the liquid smoke is the main component [36] that inhibits the growth of bacterial populations by extending the lag phase proportionally in the product, while the growth rate in the exponential phase remains unchanged except the concentration of phenol that is very high. While phenol at low concentrations only adds cell membrane permeability, so that cell metabolites will come out and inactivate the bacterial enzyme. At the concentration of 1%, phenol has function as bacteriostatic (inhibit microbial growth), whereas at higher concentrations, it acts as bactericidal (killing bacteria) [37].

The regression equation on combination of raw materials, different pyrolysis temperature at concentration of liquid smoke 0 ppb is $y = 0.2453x + 0.0552$; $r^2 = 0.0996$, liquid smoke concentration of 1 ppb $y = 0.2592x + 0.0742$; $r^2 = 0.0974$; 10 ppb concentration of liquid smoke $0.3579x + y = 2.1626$; $r^2 = 0.0577$; liquid smoke concentration of 100 ppb; $0.0642x + y = 5.7139$; $r^2 = 0.0018$; liquid smoke concentration of 500 ppb $0.5655x + y = 5.7342$; $r^2 = 0.1502$; 1000 ppb concentration of liquid smoke $0.3632x + y = 8.4759$; $r^2 = 0.0738$; and 1500 ppb concentration of liquid smoke $0.4681x + y = 15.987$; $r^2 = 0.3517$. The regression equation shows that the combination of raw materials to pyrolysis temperature at different liquid smoke concentrations indicates weak relationship to resistivity diameter of Escherichia Coli bacteria, with R2 respectively at 0.096, 0.0974, 0.0577, 0.0018, 0.1502, 0.0738 and 0.3517. The relationship on combination of raw materials with different pyrolysis temperature at liquid smoke concentration 1500 ppb indicates the highest R^2 value of 0.3517, although the correlation indicates a weak relationship. Usually antibacterial tests (resistivity zone) is performed on test bacterial isolates. Resistivity diameter zone is a bacterial sensitivity area to a substance of liquid smoke at different concentrations as indicated by the clear area around the added liquid smoke area. The larger diameter that is formed, the greater influence that liquid smoke can provide. Liquid smoke resistivity zone as combination of raw materials with different pyrolysis temperature illustrate their different resistance response, resistivity zone in this case uses E. coli that is classified as Gram negative short rod-shaped having a length about 2μm, diameter 0.7 μm, width 0, 4 to 0.7 μm and has anaerobic facultative characteristic, E. coli forms circular, convex, and smooth with a real edge colonies Escherichia Coli [38].

Antibacterial test (resistivity zone) is performed on testing bacterial isolates. Resistivity diameter zone is a bacterial sensitivity area to a substance of liquid smoke at different concentrations (0 ppb, 1 ppb, 10 ppb, 100 ppb, 500 ppb, 1000 ppb and 1500 ppb) as indicated by the clear area around the added liquid smoke area, The larger diameter that is formed, the greater influence of liquid smoke can provide. Liquid smoke resistivity zone as combination of raw materials with different pyrolysis temperature illustrate their different resistance response. Resistivity zone, in this case uses E. coli, is classified as Gram-negative short rod-shaped having a length about 2μm, diameter 0.7 μm, width 0, 4 to 0.7 μm and has anaerobic facultative characteristic, E. coli forms circular, convex, and smooth with a real edge colonies Escherichia Coli [38].

The antimicrobial activity of liquid smoke is caused by phenol and acid components. Liquid smoke contains acid and its derivatives (formate, acetate, butyrate, propionate, and methyl ester), alcohol (methyl,

ethyl, propyl, alkyl, and isobutyl alcohol), aldehydes (formaldehyde, acetaldehyde, furfural and methyl furfural), hydrocarbons (Silene, cumene, and cymene), ketones (acetone, methyl ethyl ketone, methyl propyl ketone, and ethyl propyl ketone), phenol, pyridine, and methyl pyridine [24]. Phenols and acids are compounds that act as antimicrobial [25]-[32]. Phenol and its derivatives can act as bacteriostatic or bactericidal because it has the ability to inactivate essential enzymes, coagulate proteins SH group and the NH group [33].

The antimicrobial activity mechanism of phenol and its derivatives include reaction with cell membranes that causes increased permeability of cell membranes and results in loss of cell contents [34], inactivation of essential enzymes and the destruction or inactivation of functional genetic material. The higher concentration of phenol, it will further precipitate all proteins cells, conversely, the lower concentration will further effectively inhibit the enzymes essential. According to [39] that liquid smoke contains phenolic compounds which in addition to contributing smoke flavor, also has antioxidant and bactericidal action on food.

IV. Conclusions

1. The biggest resistivity diameter of Escherichia Coli bacteria occurs on the interaction treatment of cinnamon raw materials with pyrolysis temperature of 200±10°C at 14.176 mm/ppb and simultaneously regression line shows weak correlation with R^2 at values 0.0401.
2. The biggest resistivity diameter of Escherichia Coli bacteria occurs on the interaction treatment of cinnamon raw materials with liquid smoke concentration of 1500 ppb at 20,500 mm/ppb and simultaneously the regression equation shows a strong relationship with R2 values of 0.9072, it means that 90.72% resistivity diameter of Escherichia Coli bacteria is affected by treatment combination of cinnamon raw material with concentration of 1500 ppb.
3. The biggest resistivity diameter of Escherichia Coli bacteria occurs on the interaction treatment of liquid smoke concentration 1500 ppb at pyrolysis temperature of 300±10°C of 21.151 mm/ppb and the regression equation shows a strong relationship with R2 values of 0.8567, it means that 85.67% resistivity diameter of Escherichia Coli bacteria is affected by combination treatment of liquid smoke concentration at 1500 ppb on pyrolysis temperature of 300±10°C.
4. The biggest resistivity diameter of Escherichia Coli bacteria occurs on the interaction treatment of cinnamon raw materials at the pyrolysis temperature of 400±10°C in the liquid smoke concentration of 1500 ppb at 22.29 ppb and the regression equation shows a weak correlation with R2 values of 0.3517.

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