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Antimicrobial activity of coconut shell liquid smoke

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Abstract. Coconut shell liquid smoke is produced from the pyrolysis and condensation of smoke from the burning process of coconut shell. It is known to have considerably high content of polyphenol. Beside acting as antioxidant, polyphenol is also a good antimicrobial. This research was conducted in order to study the antimicrobial activity of coconut shell liquid smoke. Coconut shell liquid smoke used in this study was produced from three different processing stages, which obtained three different grades of liquid smoke (grade 1, 2 and 3). Each sample of coconut shell liquid smoke was extracted using ethyl alcohol and petroleum ether. The extract was then analyzed for its antimicrobial activity against *S. aereus, E. coli* and *C. albicans* using well diffusion method. Total phenol and microbial microscopic structure of the liquid smoke were also examined. The results showed that there was influence of coconut shell liquid smoke on the inhibition of *S. aureus, E. coli* and *C. albican* growth. This fact was marked by the forming of clear area surrounding the well on the dish agar media. The highest percentage of inhibition showed by the extract of grade 3 coconut shell liquid smoke. This may be explained by the highest total phenol content in grade 3 liquid smoke. Microscopic examination showed that there was a breakage of microbial cell walls caused by the antimicrobial property of the liquid smoke. It was concluded that coconut shell liquid smoke was beneficial as antimicrobial agent, and while all grades of liquid smoke contains polyphenol, the content was influenced by the processing stage and thus influenced its level of microbial growth inhibition.

1. Introduction
Coconut shell is a part of coconut and not commonly utilized into high value products. Traditionally coconut shell is burned and used for cooking. This practice may result in dangerous waste and increase air pollution [1]. Pyrolisis, followed by condensation, can produce high quality liquid smoke from coconut shell. Coconut shell chips is smoldered and then condensation is carried under limited oxygen to obtain liquid smoke [2].

Coconut shell liquid smoke is known to be a good food preservation agent, substituting formalin, due to its antimicrobial and antibacterial activities, thus has inhibitory effects on pathogens [3,4,5]. Antimicrobial and antioxidant compounds presenting in coconut shell liquid smoke are aldehyde, carboxylic acids and phenols [6,7].

Despite these potential in coconut shell liquid smoke, there is reluctance in using it, especially for certain products where odour, flavor and color are easily affected. For raw food, these qualities may be recovered by cooking and other handling process. Other products may not have this advantages. Therefore, refining process is often done to liquid smoke in order to reduce the undesirable odour, flavor and color [2]. Refined liquid smoke therefore is more flexible in applications to various food or non food products, compared to non refined liquid smoke [8].

There are three grading of coconut shell liquid smoke, i.e. grade 1, 2 and 3. The difference between each grade lies on the extent of filtration stages. The more refined was called Grade 1 and the less refined was Grade 3. Surely, Grade 1 has the best characteristics for consumers convenience and liking, while Grade 3 may posses some undesirable characteristics, although these characteristics were not necessarily less beneficial. Grade 1 is very slightly yellowish and has the highest clarity compared to the other grades (Figure 1). It is more commonly used as food preservatives for meatball, noodle, sausage, tofu, etc. Grade 2 is yellowish and usually used as antioxidant and antimicrobial agent, while Grade 3 is very dark with sharp odor, often used as wood preservatives, rubber coagulant and odor absorber.

Figure 1. Coconut shell liquid smoke from different grades.

The growing interest in research and industrial world encouraged a great attention on evaluation of antimicrobial activity. Various methods have been developed for this purpose. One of the recommended methods is the agar well diffusion method. This method is widely used to evaluate the antimicrobial activity of plants or microbial extracts [9,10].

The objective of this research was to evaluate the antiumicrobial activity of coconut shell liquid smoke produced from three different processing stages.

2. Material and Method

2.1. Coconut shell liquid smoke

Coconut shell liquid smoke (CS-LS) with three different grades were obtained from Indonesia Liquid Smoke, Ciracas, East Jakarta. It was produced by processing coconut shell in furnace with 400-500^{o}C for 1-2 hours and followed by condensation of the smoke (Figure 2).

The produced CS-LS from the process above was labeled as Grade 3 CS-LS. Grade 2 was produced from distillation and filtration of the condensed smoke using zeolite. Grade 1 was the result of further filtration using activated carbon.

Zeolite was prepared by calsination at 400^{o}C for 4 hours. Afterwards, it was activated using KOH solution (1N) for 24 hours and centrifuged (1500 rpm, 5 minutes) and oven-dried at 40-60^{o}C for 24 hours. Meanwhile, activated carbon was prepared using $CaCl_2$ (25%) through the same process.

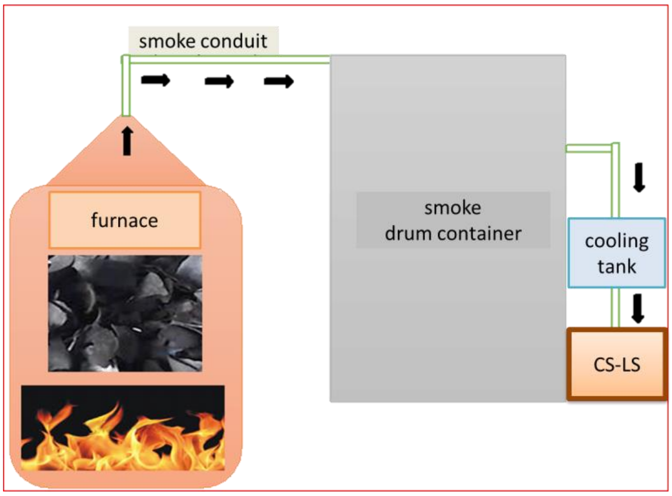

Figure 2. Scheme of coconut shell liquid smoke production.

2.2. Agar well diffusion
Agar well diffusion method was conducted by a modified method of Balouiri et al. [11]. Agar plate surface was inoculated by spreading a volume of microbial inoculum over the its entire surface. A hole with a diameter of 6-8 mm was then punched aseptically with a tip, followed by a volume of 20-100 µL of the antimicrobial agent with the concentration of 25, 50, or 75% being dripped into the well. Then, the plates of agar were incubated in 37^{o}C for 24 hours. The growth of the microbial strain was evaluated by observing the formation zones and the diameter was measured in mm. Microbial strains tested were *S. aureus, E. coli* and *Candida sp.*

2.3. Total phenolic content
The total phenolic content of CS-LS was determined by Folin-Ciocalteu method as was done by Kaur and Kapoor [12]. 200 µL of sample (1 mg/mL) were made up to 3 mL with distilled water, followed by the addition of 0.5 mL of Folin–Ciocalteu reagent and the mixture were mixed thoroughly for 3 min. 2 mL of 20% (w/v) sodium carbonate was then added prior to 60 min storage in the dark. Using spectrophotometer, absorbance was measured at 650 nm. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of gallic acid equivalent per g dry weight.

2.4. Physicochemical characteristics
Color of CS-LS was evaluated using Chromameter. Chromameter was calibrated using white standard material. The color was valued by Hunter color system (L, a, and b value) with white as standard color (L1, a1, b1).

Clarity was analyzed using Spectrophotometer. Distilled water in cuvet was used as 100% clarity control. CS-LS sample in other cuvet was evaluated with 420 nm wavelength.

pH was measured using pHmeter. The electrode of pHmeter was simply dipped into the CS-LS sample until the instrument showed the measurement result.

3. Results and Discussion
The antimicrobial effects of CS-LS as shown by the agar well diffusion analysis results were only significant for the growth of *Staphylococcus aureus* and *E. coli*. There was no antimicrobial effect was shown for the growth of *Candida sp.* This proved that antimicrobial activity in CS-LS was more effective on bacteria then on yeast. Moreover, it was more obvious on the test for *S. aureus* where all samples of CS-LS showed inhibitory effects, leading to conclusion that CS-LS was more effective to inhibit the growth of gram-positive bacteria. Some studies reported that gram positive bacteria were more sensitive than gram negative ones to some plants extract [13]. On gram-positive bacteria, all

grades of CS-LS with each concentration provided good inhibition effect (shown as the forming of clear area around the wells) (Figure 3).

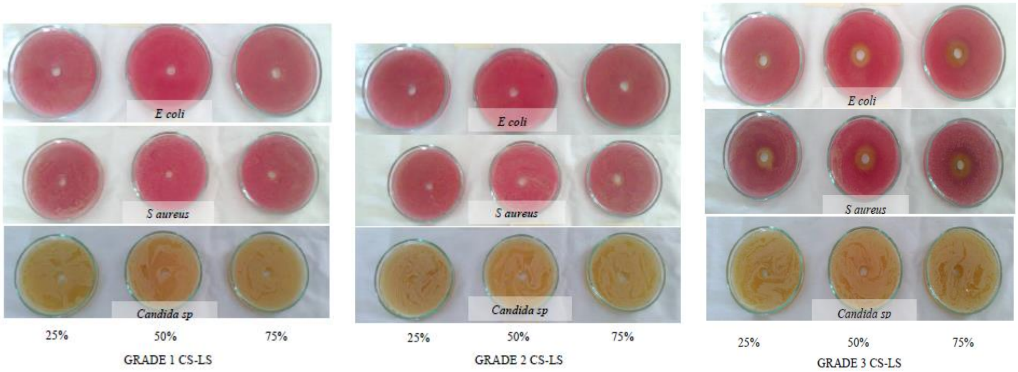

Figure 3. Inhibitory effects of coconut shell liquid smoke (CS-LS) from three different grades and three different concentration on *E coli, S aureus* and *Candida sp*.

Results also showed that higher concentration of liquid smoke gave better inhibitory effect to the growth of bacteria. Antimicrobial effect of liquid smoke was contributed by its phenolic content. The higher total phenolic content in CS-LS, the better its antimicrobial activity. This fact was demonstrated in this research. Table 1 showed that Grade 3 CS-LS had the highest total phenolic content (Table 1) and it was correlated with the best inhibitory effect on microbes. Filtration using zeolite and activated carbon can decrease phenol content [14]. Grade 3 CS-LS did not undergo filtration process, thus contains more phenol.

Polyphenols are bioactive molecules. Biological activities are related to the molecules structures; by their hydroxyl groups or by phenolic ring, phenolic compounds have the capacity to link with proteins and bacterial membranes to form complexes [15]. Phenolic compounds, such as phenol, 2-methoxylphenol (guaiacol), 3,4dimethoxylphenols, and 2-methoxy-4-methyl phenol are prominent in CS-SL and play a major contribution to antibacterial activity [16]. The antimicrobial mechanism from phenolic compounds on gram positive bacteria might be described as disrupting cell wall, increasing cell membrane permeability, and then leading to the leakage of cell constituents [13].

Table 1. Physicochemical properties of coconut shell liquid smoke

Parameters	Coconut shell liquid smoke		
	Grade 1	Grade 2	Grade 3
Total phenolic content (%)	0.18±0.004	0.30±0.002	1.5±0.1
Lightness (L)	95.76	93.01	29.87
a	1.97	-1.26	39.97
b	5.50	20.82	47.92
Clarity (%)	93.70	64.23	0.00
pH	2.79	3.12	2.76

Zeolite has adsorber ability due to its porous structure, allowing tar and benzopyrene compounds in liquid smoke to be caught in its pores. Zeolit can absorb a considerable amount of molecules with the same or smaller size than zeolite's pores [17].

Activated carbon has a vast carbon surface and porous structure due to its granules form. Aromatic compounds with the same or smaller molecules size than its pores can be adsorbed during filtration. This mechanism allows liquid smoke to lost its smoke aroma and flavor and making it more acceptable as food preservatives.

Table 1 also presented the different physicochemical properties of CS-LS based on their grades. Grade 1 had the highest lightness (L value) and clarity compared with the other grades. Grade 3 had a considerably dark and cloudy appearance. These properties alone could be a determinant values in the utilization of each grade of CS-LS. As mentioned above, Grade 1 is more common as food preservatives, probably because of its better aroma and appearance, Grade 2 is used as antimicrobial agent and Grade 3 for wood preservatives and rubber coagulant, where aroma and appearance are not ranked very high in the selection of material.

4. Conclusions

CS-LS was effective to inhibit the growth of gram-positive bacteria due to its total phenolic compound. Higher total phenol gave better inhibition effect. The filtration process can reduce the phenol content of CS-LS.

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