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POTENTIAL OF LIQUID SMOKE IN INHIBITING THE GROWTH OF PATHOGENIC BACTERIA: *A MINI REVIEW*

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ABSTRACT

The objective of this study was to perform a comprehensive evaluation of the application of liquid smoke as a possible antibacterial agent. The studies were identified by conducting searches on multiple electronic databases, specifically Semantic Scholar, PubMed, and Google Scholar, from 2013 to 2024. The keywords used included 'antibacterial of wood vinegar, antibacterial of pyroligneous extract, antibacterial of pyroligneous acid, antibacterial of wood distillate, antibacterial activity of liquid smoke, and aktivitas antibakteri asap cair (in Bahasa Indonesia)'. The results indicate that liquid smoke has the capacity to serve as a natural antibacterial agent against both Gram positive and Gram-negative microorganisms. The variation in antibacterial susceptibility may arise from the composition of chemical compounds present in liquid smoke, the raw materials employed in its production, the temperature of pyrolysis, and the specific testing methodology employed. This review will be useful to the industrial and scientific communities in the food science, pharmaceutical, and technology fields.

Keywords: Antibacterial, Chemical Compounds, Raw Materials, Pyrolysis, Wood Vinegar

INTRODUCTION

Exposure to various microorganisms such as viruses and fungi can lead to infections. Pathogens that cause damage to human organs and are commonly associated with infectious agents, such as bacteria, often result in increased morbidity and mortality. Infection-causing bacteria can be Gram-negative or Gram-positive. Bacterial infections can be treated through the use of antibacterial drugs, but the use of antibacterial drugs is currently used frequently, causing drug resistance (Yulstiani *et al*., 2018). Along with the increasing rate of bacterial resistance to drugs, it is necessary to develop new antibacterial drugs using medicinal plants that can minimize the side effects of using antibacterial drugs.

Liquid smoke, also referred to as pyroligneous acid or wood vinegar, is the product of a high-temperature biomass carbonisation process in the absence of oxygen or minimal oxygen (pyrolysis) (Adfa *et al*., 2017). Liquid smoke has been widely used as a food preservative, bioinsecticide, herbicide, and various other purposes, due to its unique chemical composition (Salamah & Jamilatun, 2017). Liquid smoke from various biomasses has also been reported to inhibit the growth of bacteria and fungi.

Up to 200 different compounds can be found in liquid smoke, including phenols, ketones, furans, aldehydes, pyrans, alcohols, and organic acids. Liquid smoke functions as an anti-inflammatory, antibacterial, and antioxidant in a range of applications. Researchers from all over the world are becoming more interested in the pyrolysis bio products of wood and other biomass due to their variety of applications; some of these studies concentrate on the bio products' antibacterial and other characteristics. Liquid smoke has been shown by various researches to have antibacterial activity against a variety of pathogenic bacteria. The review by Brustolin *et al*.(2024) provides an excellent summary of the use of liquid smoke and its antibacterial characteristics in food systems, particularly in fish. This review expands on the information presented in and provides a more detailed discussion on the effectiveness of liquid smoke as an antibacterial agent against pathogenic bacteria, including the raw materials used in its production, the composition of chemical compounds presents in liquid smoke, the temperature of pyrolysis, and the specific testing methodology employed.

METHOD

This research began with a literature search on the antibacterial activity of liquid smoke. This literature search technique was carried out by searching data on the Semantic Scholar, PubMed, and Google Scholar databases between 2013 and 2024 using the keywords antibacterial of wood vinegar, antibacterial of pyroligneous extract, antibacterial of pyroligneous acid, antibacterial of wood distillate, antibacterial activity of liquid smoke, and aktivitas antibakteri asap cair (in Bahasa Indonesia). There are 25 articles analyzed, 20 published by international journal, and 5 by Indonesian national journals. The related data that was collected is then tabulated, analyzed and discussed in this study (Wibowo *et al*., 2023).

RESULTS AND DISCUSSION

Base Material/Biomass

Liquid smoke is essentially an acidic product with a pH ranging from 2.5 to 3.6 depending on the chemical composition of the biomass source (Rahmat *et al*., 2014). This acidity is caused by organic acids. The most common organic acid reported from liquid smoke is acetic acid, with varying concentrations. Liquid smoke also acts as a source of valuable chemicals and provides smoky flavour and antimicrobial protection to foods. Based on the literature review, as can be seen in Table 1, most researchers have reported the potential of liquid smoke as an antibacterial agent, along with information on the biomass source, pyrolysis temperature, type of bacteria and the ability to inhibit bacterial growth or the ability to kill bacteria.

Based on Table 1, liquid smoke can be synthesised from various biomasses, including: *Cinnamomum burmannii* wood waste, *Hevea braziliensis* fruit shells,

Eucommia ulmoides (olive) branches, cashew seed shells, cacao fruit shells, eucalyptus (*Melaleuca leucadendra)*, teak wood (*Tectona grandis*), *Litchi chinensis,* palm shells, coconut shells (*Cocos nucifera L.*), *Dimocarpus longan* wood, rice husk (*Oryza sativa*), Coconut shell (*Cocos nucifera L.*), *Castanea sativa* Mill., *Eucalyptus urograndis* wood, *Anacardium occidentale* nut shells and *Cocos nucifera* shells*,* pine wood sawdust (*Pinus sylvestris*), bamboo stems (*Gigantochloa apus*)*, Bambusa vulgaris*, *Melaleuca leucadendron* twigs, as well as a mixture of several biomasses such as a mixture of *Eucalyptus urograndis* and *Mimosa tenuiflora* wood, a mixture of pine-cypress wood-particles, and a mixture of sawdust and cocoa pods *(Theobroma cacao).* In addition to pyrolysis temperature, the biomass source plays an important role in the chemical composition of the liquid smoke produced, depending on the amount of sellulose and lignin it contains (Kawamoto, 2017; Lin *et al*., 2009). The variety of raw materials and liquid smoke production methods create complicated chemical components with different structures and reactivity.

Pyrolysis

A method to employ lignocellulose biomass is the pyrolysis process. Pyrolysis is the thermal degradation of biomass materials in the absence or minimal presence of oxygen, resulting in the production of solid and liquid products such as charcoal, edible oil, or wood vinegar. Wood vinegar, sometimes known as liquid smoke, is a liquid generated from the carbonization or pyrolysis of waste and lignocellulose biomass. Rapid depolymerisation generates liquid smoke while concurrently degrading hemicellulose, cellulose, lignin, and organic components. Biopolymers decompose into smaller molecules under elevated temperatures and restricted moisture. The smoke generated from the carbonization process is sent into a condenser, where it transforms into a liquid, namely liquid smoke (Kawamoto, 2017; Shurong *et al*., 2017).

Pyrolysis usually occurs at temperatures above 300 °C and lasts 4-7 hours. The process consists of several reactions: decomposition, oxidation, polymerization, and condensation. The end result of biomass pyrolysis is charcoal (bio char), gas that can be condensed into liquid smoke/vegetable oil, and non-condensable. Pyrolysis involves losing water from biomass at 200 °C, converting hemicellulose to 355 °C, decomposing cellulose at 240 to 410 °C, and decomposing lignin at 150 to 900 °C (Wibowo *et al*., 2023). Table 1 shows that liquid smoke was synthesised at temperatures ranging from 100 to 600 °C. That is, at 75 °C, from 90-120 °C, 150 °C, 150-300 °C, 250 °C and 260 °C, 300°C, 525-530 °C, 400 °C, from 200 to 600 °C, from 270-300 °C, 300-330 °C, 480-510 °C, from 400 to 500 °C, at 450 °C, 475 °C, 300 °C, 400 °C, 340 °C, and 380 °C.

Chemical Component of Liquid Smoke

During pyrolysis, cellulose undergoes hydrolysis, yielding glucose, acetic acid, water, and some phenol. Pyrolysis of lignin yields phenol compounds and derivatives, while high temperatures result in the production of tar. During pyrolysis, hemicellulose yields furfuran, furan, and carboxylic acid. The pyrolysis process can produce a variety of compounds with different compositions; however, the chemical and physical properties of liquid smoke are thought to be influenced by the raw material type as well as the pyrolysis operation conditions (Budaraga *et al*., 2026b).

Table 1 presents the chemical constituents of liquid smoke derived from diverse biomasses, mostly comprising carboxylic acids, phenolics, furfural, and water. Additional components include aldehydes, ketones, alcohols, benzene and its derivatives, fatty acids, and methylamine. The principal carboxylic acid present is acetic acid, while the predominant phenolic compounds include 2-methoxyphenol (guaiacol), 2,6-

dimethoxyphenol (syringol), 3-methoxy-1,2-benzenediol, phenol, 1,2-benzenediol (catechol), 4-methylcatechol, 3-methylcatechol, 4-methoxyphenol, and 1-hydroxy-3-methylbenzene (m-creosol).

Type of Test Bacteria

Liquid smoke from 25 types of biomass (Table 1) prevent the growth of several types of pathogenic bacteria, including Gram-positive and Gram-negative bacteria. Bacteria that can be inhibited in this literature review are: *Acinetobacter baumannii, Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *B. cereus*, *Multi-antibiotic-resistant strains* against *E. coli, P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 25923. *Listeria monocytogenes, Micrococcus luteus, P. fluorescens, Salmonella typhimurium, Serratia marcescens, S. epidermidis, Streptococcus mutans,* methicillin-resistant *S. aureus* (CRP41 and CR010). *Enterococcus faecalis, Salmonella choleraesuis, Porphyromonas gingivalis, Enterobacter cloacae*, *Lactococcus garviae*, *Proteus vulgaris*, *Vagococcus salmoninarum*, *Listonella anguillarum, Aeromonas salmonicida*, *Streptococcus agalactiae, Salmonella enteritidis,* and *S. enterica* subsp. enterica serovar Typhimurium. The chemical components of liquid smoke, the amount of liquid smoke applied, the antibacterial activity test method, and the liquid smoke purification technique are some of the variables that affect the liquid smoke's capacity to prevent the growth of test bacteria (Suresh *et al*., 2019).

Antibacterial Assay and Concentrations Tested

The antibacterial activity of liquid smoke in vitro can be evaluated or screened using a variety of laboratory procedures. The most common and basic methods are disk diffusion, well diffusion, and agar dilution. Agar disc diffusion testing, introduced in 1940, is an authoritative method used in clinical microbiology laboratories for antimicrobial susceptibility testing. The Clinical and Laboratory Standards Institute publishes recognized standards. This method accurately tests for pathogenic bacteria like Streptococcus, *Haemophilus influenzae*, *H. parainfluenzae*, *Neisseria gonorrhoeae*, and *N. meningitidis.* It uses specific culture media, incubation conditions, and interpretative criteria for zones of inhibition. To perform this test, agar is inoculated with a standard microbe. Filtered paper discs (about 6 mm in diameter) containing the necessary concentration of test material are then placed on the agar surface (Balouiri *et al*., 2016).
The agar-well diffusion method is commonly used to test the microbial activity of plant extracts and other materials. Similar to the disc diffusion approach, the agar plate surface is infected with microbial inoculum before being aseptically perforated with a cork borer 6-8 mm in diameter. A total of 20-100 µL antimicrobial drugs at the specified concentration are poured into the wells, and the agar plate is incubated under proper conditions, depending on the test microorganisms. The antimicrobial drug diffuses into the medium, inhibiting the growth of the tested strain (Balouiri *et al*., 2016).

The most suitable technique for determining MIC values is dilution, as it allows for the estimation of the concentration of the examined antimicrobial agent in the agar (agar dilution) or broth medium (macro dilution or micro dilution). In vitro antimicrobial activity against bacteria and fungi can be quantitatively assessed using either the broth or agar dilution method. The minimum inhibitory concentration (MIC) value is the lowest concentration of the assay antimicrobial agent that prevents the visible growth of the microorganism under assessment (Balouiri *et al*., 2016). Minimum Bactericidal

Concentration (MBC) refers to the lowest concentration of an antimicrobial that will kill 99.9% of the initial bacterial inoculum (Kłodzińska *et al*., 2018).

Table 1 shows a wide range of liquid smoke antibacterial test concentrations, beginning with the lowest concentration of liquid smoke from *Cinnamomum burmannii* wood waste, which is 0; 1; 10; 100; 500; 1000; and 1500 ppm. Other liquid smoke test concentrations are indicated in percentage v/v, ranging from 1; 2; 2.5; 3; and 4% (v/v) to 5, 10, 25, 50, 75, and 100%. The MIC and MBC of the liquid smoke presented in Table 1 fluctuated based on the chemical constituents of the liquid smoke and the bacterial strains tested. The minimal MIC was observed with a combination of pine, spruce, and fir wood particle liquid smoke (no purification with distillation or extraction) at concentrations of 0.3125-0.625% v/v against *Escherichia coli*, *Enterobacter aerogenes*, *Listeria monocytogenes*, and *Enterococcus faecalis*, respectively (Suresh *et al*., 2019). The MBC commences at 3.125%.

Investigation of the Antimicrobial Properties of Liquid Smoke and Its Correlation with Biomass Type, Pyrolysis Temperature, and Chemical Components

Liquid smoke from *Eucommia ulmoides olivers* branches was examined by Hou *et al*. (2018) for its antibacterial properties at four different temperature ranges: 90-120°C, 270-300°C, 300-330°C, and 480-510°C. GC-MS results revealed that phenols, ketones, aldehydes, alcohols, organic acids, and benzene were the main components of liquid smoke from the branches of *E. ulmoides olivers*. Antibacterial activity tests were conducted against *E. coli*, *E. aerogenes*, *S. aureus, B. cereus* and *B. subtilis*. The results indicated that against the five test pathogens, all liquid smoke samples exhibited antibacterial activity. For three of the five bacterial species, liquid smoke (300–330°C) showed the strongest antibacterial activity: *E. aerogenes* (24.50 mm) > *E. coli* (21.00 mm) > *B. subtilis* (19.67 mm). The most powerful antibacterial activity was seen in liquid smoke (240–270 °C), with inhibition zones of 32.00 mm for *S. aureus* and 23.50 mm for *B. cereus*. The antibacterial activity of liquid smoke (240–270°C) against *S. aureus* was significantly higher than that of the tetracycline positive control (17.09 mm) and more pronounced than that observed for the other four microorganisms.

Tobing *et al.* (2021) tested the antibacterial activity of coconut shell liquid smoke (*Cocos nucifera* L.) against *E. coli*. The pyrolysis temperature used was 150-300°C and then allowed to stand for 24 hours to separate the tar. The test results indicate that coconut shell liquid smoke can suppress the growth of *E. coli*. The most extensive zone of inhibition occurs at a concentration of 50%, measuring 16.66 mm. 9.33 mm was the smallest inhibition zone observed at a 10% concentration, while no clear zone was observed at a 5% concentration. The MIC was also determined at a concentration of 10% in this study.

The antibacterial properties of *Anacardium occidentale* nut shell (ANOLIS) and *C. nucifera* L. shell liquid smoke (CONULIS) against *E. coli* bacteria were also examined by Pasaribu *et al*. (2022). The results indicated that the virginiamycin positive control's (8.5 mm) inhibition zone was comparable to that of ANOLIS 25-35% (9 mm) and CONULIS 5% (9 mm). According to this study, the bactericidal activity of CONULIS 5% and ANOLIS 25–35% is nearly identical to that of virginiamycin, which was employed as a positive control promoter. The antibacterial properties of liquid smoke from coconut shells against *E. coli* and *S. aureus* were examined by Kailaku *et al.* (2017). Pyrolysis was conducted at a temperature of 400–500°C, and it was followed by distillation and filtering. In comparison to Gram-negative bacteria (*E. coli*), the findings indicated that coconut shell liquid smoke was more effective against Gram-positive bacteria (*S. aureus*).

Chukeatirote & Jenjai (2018) tested the antibacterial activity of *Dimocarpus longan* liquid smoke. The main components in this liquid smoke are n-hexadecanoic (palmitic), tetradecanoic (myristic), and 9-octadecenoic (oleic) acids which are known from GC-MS results. Antibacterial activity test of 100% liquid smoke was conducted against 14 bacterial strains. Based on the diffusion test, the potent *D. longan* liquid smoke showed a broad antibacterial spectrum against both Gram-positive and Gram-negative bacteria based on the diffusion test, with inhibition zones spanning from 10.44 to 19.56 mm. According to the research's results, susceptibility was ranked overall as follows *S. aureus* > *E. coli* > *B. cereus* > *B. subtilis* > *S. epidermidis*.

The antibacterial effect of bamboo liquid smoke (*Gigantochloa apus*) against the growth of *S. aureus* and *S. epidermidis* was investigated by Siregar *et al.* (2022). The MIC and MBC of *G. apus* liquid smoke against *S. aureus* and *S. epidermidis* are obtained at a concentration of 5% based on the results of the tests that have been conducted. *G. apus* liquid smoke at a concentration of 5% can inhibit and kill *S. aureus* and *S. epidermidis*.

Budaraga *et al*. (2016) evaluated liquid smoke derived from cinnamon against *E. coli* ATCC 11778. The pyrolysis of liquid smoke was conducted at 400°C, subsequently followed by distillation and decantation. The variation test results indicated that various purification methods for liquid smoke significantly influenced its inhibitory impact on *E. coli*, however the interaction between purification treatment and liquid smoke concentration did not significantly affect the inhibition diameter. In 2019, the researchers evaluated the inhibitory effect of liquid smoke derived from cocoa pods on *E. coli* and *S. aureus*. Liquid smoke derived from cocoa pods at a concentration of 10% demonstrated the most effective inhibition of *S. aureus* and *E. coli* growth. The liquid smoke derived from cocoa pods at a concentration of 10% exhibited an inhibitory zone of 25.1 mm against *E. coli* and 32.6 mm against *S. aureus*. *E. coli* exhibited greater resistance than *S. aureus*.

De Souza Araújo *et al*. (2018) tested the antibacterial activity of liquid smoke of *Eucalyptus urograndis* and *Mimosa tenuiflora* wood slices. Pyrolysis was carried out to a temperature of 450°C, followed by vacuum bi-distillation at a pressure of 5 mmHg. GC-MS results showed that the main components of both liquid smokes are furfural and phenol. Antibacterial activity test was conducted against *E. coli*, multi-antibiotic-resistant strains, *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853. The results indicated that the smallest concentration of 20% could inhibit the growth of all test bacteria. At a 20% concentration, the inhibitory zone values of *E. urograndis* liquid smoke were 11.3 mm for *P. aeruginosa* and 12.3 mm for *S. aureus*. In contrast, the inhibitory zone values of *M. tenuiflora* liquid smoke at 20% concentration were 11 mm and 13 mm against *P. aeruginosa* and *S. aureus*.

Suresh *et al*. (2019) determined the MIC value of liquid smoke from a mixture of pine, spruce, and spruce wood particles against 5 pathogenic bacteria namely *E. aerogenes* ATCC 13048, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 15442, *E. faecalis* ATCC 29212 and *L. monocytogenes* ATCC 191110. Liquid smoke pyrolysis was carried out at 475°C and then followed by GC-MS to analyse the compound content contained in the liquid smoke. From the GC-MS results, it was found that the main contents of the liquid smoke mixture of pine, fir, and fir wood particles were water 45%, acetic acid 3.9%, catechol 8.72%, 4-methylcatechol 7.41%, 3-methylcatechol 3.24%, and 4-ethylbenzenediol 3.54%. Liquid smoke was tested for its antibacterial properties at neutral pH (7.0) and acidic pH (3.7). Against five strains of pathogenic bacteria, neutralized liquid smoke demonstrated greater antibacterial activity than acidic liquid

smoke. For *E. coli*, *E. aerogenes*, *P. aeruginosa*, *L. monocytogenes*, and *E. faecalis*, the MIC obtained with neutralized liquid smoke was 0.3125% (v/v), 0.3125% (v/v), 0.625% (v/v), 0.3125% (v/v), and 0.3125% (v/v), respectively.

Gama *et al.* (2022) tested the antibacterial activity of *E. urograndis* wood liquid smoke on 4 bacterial strains namely *P. aeruginosa (*ATCC 27853), *S. enteritidis (ATCC* 13076), *S. aureus (ATCC* 25923), *S. agalactiae* (CEPA CLINICA) with different pH. Pyrolysis was carried out to 450°C and then stored for 2 weeks to separate the tar and continued with bi-distillation. The inhibition pattern as a function of concentration and pH of liquid smoke was different for each microorganism. The MIC obtained at the lowest pH (2.5) was 3.12% for *P. aeruginosa* and *S. enteritidis*. At the lowest pH, however, the MIC for *S. aureus* and *S. agalactiae* was 6.25%. For *P. aeruginosa, S. enteritidis, S. aureus,* and S*. agalactiae*, the MICs were 25%, 50%, 50%, and 50% at the highest pH (7.5), respectively. At lower pH levels, the concentration required to inhibit microbial growth is lower and as the pH approaches neutral, the concentration required to achieve the same effect is higher.

Table 1. Antibacterial Activity Of Liquid Smoke From Several Biomass Sources

Liquid Smoke Source	Pyrolysis Temperature	Major Chemical Components	Bacteria Assayed	Methods	Test Concentrations/MIC and MBC	References
Litchi chinensis (no part information)	100 – 600°C	2-methoxyphenol (guaiacol) 12,36%; 2,6-dimethoxyphenol (syringol) 29,54%; 3,5-dimethoxy-4-hydroxytuluene 11,07%	*A. baumannii, E. coli*, *P. aeruginosa*, *S. aureus*	Microdilution	MIC ranging: 0.95-3.80 µL/100 µL MBC ranging: 1.90-3.80 µL/100 µL	(Yang *et al*., 2016)
Cinnamomum burmannii wood waste	400°C, followed by distillation/activated charcoal/decantation	No information	*E. coli* ATCC 11778	Disk diffusion	0; 1; 10; 100; 500; 1000; and 1500 ppm	(Budaraga *et al*., 2016)
Hevea braziliensis fruit shells	No pyrolysis temperature information, the liquid smoke obtained is then distilled in 2 stages	No information	*E. coli*	Disk diffusion	5; 10; 25; 50; 75; and 100%	(Oktarina *et al*., 2017)
Palm kernel shell	Pyrolysis at 200-600°C, and extracted using ethyl acetate	Liquid smoke extracted with ethyl acetate contained 71.34% phenol, 10.54% organic acid 4-hydroxybenzoic acid, 1.5% ketone. The main components of	*P. aeruginosa* ATCC 27853, *B. subtilis* WICC IBD-b69, *E. coli* ATCC 35218, *S. aureus* ATCC 35218	Dilution and diffusion	1000 mg/mL MIC: 1.95-3.91 mg/mL MBC: 62.5 - 125mg/mL	(Ariffin *et al.*, 2017)

		phenol were 2,6-dimethoxyphenol/syringol 10.31%, 3-methoxy-1,2-benzenediol/pyrocatechol,3-methoxy 6.59%.				
Hevea braziliensis fruit shells	No pyrolysis temperature information, liquid smoke was distilled and re-distilled at 200°C	No information	*S. aureus*	Well diffusion	5; 10; 25; 50; 75 and 100%	(Agustina *et al*., 2017)
Coconut shells	400-500°C then followed by distillation and filtration	No information	*E. coli* and *S. aureus*	Well diffusion	25; 50; and 75%	(Kailaku *et al*., 2017)
Eucommia ulmoides (olive) branch	90-120°C, 270-300°C, 300-330°C, 480-510°C	Phenols, aldehydes, ketones, alcohols, benzene , and organic acids	*E. coli*, *B. subtilis*, *B. cereus, E. aerogenes*, *S. aureus*	Disk diffusion	100%	(Hou *et al.*, 2018)
Mimosa tenuiflora and *Eucalyptus urograndis* wood	Pyrolysis to 450°C, followed by vacuum bi-distillation at 5 mmHg.	Furfural and phenol	*E. coli*, multi-antibiotic-resistant strains of *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853	Agar diffusion	100; 50; 20% (can inhibit the growth of all test bacteria at 20%)	(De Souza Araújo *et al*., 2018)
Dimocarpus longan wood	No information	Tetradecanoic acid (myristic 7.16%), n-hexadecanoic acid (palmitic 24.33%), and 9-octadecenoic acid (oleic 24.56%)	The fourteen bacterial strains used were *B. subtilis TISTR* 008, *B. cereus* TISTR 687, *L. monocytogenes* DMST 17303, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 15442, *M. luteus* TISTR 884, *P. fluorescens* TISTR 358, *S. typhimurium TISTR* 292, two methicillin-resistant *S. aureus* strains (CRP41 and CR010), *S. aureus* TISTR	Agar diffusion and dilution	100%	(Chukeatirote & Jenjai, 2018)

			1466, *S. epidermidis ATCC* 14990, two methicillin-resistant *S. aureus* strains (CRP41 and CR010), *S. mutans* DMST 26094, and *S. marcescens* TISTR 1354.			
Cocoa pods shells	No information	No information	*E. coli* and *S. aureus*	Well diffusion	10; 15; 20; and 25% MIC and MBC against *E. coli* 3.125; 12.5%	(Budaraga & Putra, 2019)
A mix of pine, spruce and fir wood particles	475°C	Water 45%, acetic acid 3.9%, catechol 8.72%, 4-methylcatechol 7.41%, 3-methylcatechol 3.24%, 4-ethylbenzenediol 3.54%.	*E. aerogenes* (ATCC 13048), *E. coli* (ATCC 25922), *L. monocytogenes* (ATCC 191110), *P. aeruginosa* (ATCC 15442), and *E. faecalis* (ATCC 29212).	Microdilution	MIC ranging (0.3125-0.625% v/v)	(Suresh *et al*., 2019)
Cashew seed shell	No information	No information	*S. aureus* ATCC 25923	Disk diffusion	100; 75; 50; 25; and 12.5%	(Nurliana & Musta, 2019)
Cocoa pod shell	300°C, 340°C, and 380°C	No information	*E. coli* ATCC 25992, *S. choleraesuis* ATCC 14028, *S. aureus* ATCC 25923, and *B. subtilis* ATCC 6633.	Disk diffusion	1; 2; 3; 4; and 5%	(Desvita *et al*., 2021)
Biomass of Eucalyptus (*Melaleuca leucadendra*) and Teak Wood (*Tectona grandis*)	250°C	From eucalyptus: acetic acid 45.35%; phenol 6.53% From teak wood: acetic acid 25.35%; phenol 11.59%	*E. coli* FNCC 194, *S. aureus* FNCC 0047, and *P. aeruginosa* FNCC 0156.	Diffusion and dilution	Eucalyptus MIC: 6.25%-12.5% Tectona MIC: 3.125-25%	(Suryani *et al*., 2020)
Rice husk	No information	No information	*P. gingivalis* ATCC 33277	Dilution and diffusion	1; 2.5; 5; 7.5; 10; 12.5; 15; 17.5; 20; and 22.5%	(Arundina *et al.*, 2020)
Coconut	150-300°C,	No information	*E. coli*	Well	0; 5; 10;	(Tobing *et*

Shell (*Cocos nucifera* L.)	then allowed to stand for 24 hours to separate the tar			Diffusion	15; 20; 25; 30; 35; 40; 45; 50% (v/v) MIC 10% (v/v)	*al*., 2021)
Castanea sativa Mill.	Pyrogasification reactor 75°C	No information	*S. aureus* ATCC 25923, *E. faecalis* ATCC 29212, *B. subtilis* ATCC 6633, *M. luteus* ATCC 4698, *B. cereus* ATCC 11778, *E. coli* ATCC 23739, *E. cloacae* ATCC 13047, *S. typhimurium* ATCC 23853, *P. vulgaris* ATCC 6380, *L. garviae* DSM 6783, *P. aeruginosa* ATCC 17853, *A. salmonicida* DSM 46293, *L. anguillarum* DSM 11323, and *V. salmoninarum* DSM 6633.	Micro dilution	MIC (0.39-1.56% v/v)	(Misuri & Marri, 2021)
Eucalyptus urograndis wood	Up to 450°C and then kept for 2 weeks to separate the tar and continued with bi-distillation	No information	*S. enteritidis* (ATCC 13076), *P. aeruginosa* (ATCC 27853), *S. agalactiae* (CEPA CLINICA), *S. aureus (*ATCC 25923),	Microdilution	50; 25; 12.55; 6.25; 3.125; 1.5625; and 0.78123% (v/v) MIC: 3.12% for *P. aeruginosa* and *S. enteritidis*	(Gama *et al*., 2022)
Anacardium occidentale nut shells and *Cocos nucifera* shells	No information	No information	*E. coli*	Well diffusion	5; 10; 15; 20; 25; 35; 40; 60; 80; and 100%	(Pasaribu *et al*., 2022)
Pine wood sawdust (*Pinus*	150, 260, 300°C		*L. monocytogenes* ATCC 7644,	Well diffusion and	1; 2; 5; 10; 25% for *Salmonella*	(Riekkinen *et al*., 2022)

sylvestris)			*Salmomella Infantis*	dilution	0.1; 0.2; 1; 2; 5; 10; 25% for *Listeria* MIC: 0.83%	
Stem of bamboo tali (*Gigantochloa apus*)	No information	Hexadecanoic acid, methyl ester 15.09%, phenol 14.76%, 4-methoxyphenol (11.94%), octadecanoic acid, methyl ester (13.90%)	*S. aureus* and *S. epidermidis*	Well diffusion and dilution	20; 40; 60; 80; and 100% MIC and MBC: 5%	(Siregar *et al.*, 2022)
Rice husk	Pyrolysis at 300°C, and 400°C. Following distillation for purification, spray drying was used to turn the liquid smoke into liquid smoke powder.	Acetic acid, butanal, 2-cyclopentene, propanoic acid, 2-propanone, and phenolic compounds.	*E. coli*, *S. choleraesuis*, *B. subtilis* and *S. aureus*	Disk diffusion	No information	(Muriady *et al.*, 2022)
Sawdust and cocoa pod husks *(Theobroma cacao)*	525-530°C	Sawdut *Theobromo cacoa*: Acetic acid 64.64%, phenol 12.08%, acetone (5.78%) Cocoa Pod husk: Methylamine (37.26%), acetic acid (24.61%), Acetone (19.89%)	*S. aureus* FNCC 0047, *E. coli* FNCC 0091 and *S. typhimurium* FNCC 0150.	Microdilution	Sawdut *Theobromo cacoa* MIC: Ranging 0.39-1.56% Cocoa Pod husk MIC: Ranging 3.125-6.25%	(Suryani *et al.*, 2023)
Wood *Eucalyptus urograndis*, and *Bambusa vulgaris*	Up to 450°C, the liquid smoke is then stored for 3 months to separate the tar, then continued with 20 mmHg vacuum bi-distillation to 100-103°C.	From *Eucalyptus urograndis* was furfural 17.2%, while *Bambusa vulgaris* was phenol 15.3%, and acetic acid 12.5%.	*S. aureus*, *S. agalactiae*, *S. enteritidis*, *P. aeruginosa* and *E. coli*	Agar dilution	50; 25; 12.5; 6.25; 3.125; 1.5625; and 0.78123%	(Gama *et al.*, 2023)
Eucalyptus branches	Three fractions	Each fraction of liquid smoke	*B. subtilis* ATCC 19659, *L.*	Agar diffusi	No informatio	(Mansur *et al.*, 2021)

(*Melaleuca leucadendron*) twigs and rice husk (*Oryza sativa*)	were recovered by pyrolysis at 500°C followed by vacuum evaporation of the liquid smoke at low temperatures (40°C, 50°C, and 60°C).	derived from eucalyptus twigs and rice husks contains acetic acid, phenol, guaiacol, and m-creosol.	*monocytogenes* ATCC 7644, *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *S. typhimurium* ATCC 14028.	on	n

Oktarina *et al.* (2017) tested liquid smoke from *Hevea braziliensis* fruit shells against *E. coli*. The inhibition zone value of *Hevea braziliensis* fruit shell liquid smoke against *E. coli* at concentrations of 5%, 10%, 25%, 50%, 75% and 100% were 3.7 mm; 4.3 mm; 4.7 mm; 5.3 mm; 7.8 mm; and 11.3 mm, respectively. In the same year Agustina *et al.* (2017) also conducted research with the same liquid smoke against *S. aureus*. The well diffusion method was used to investigate the antibacterial activity of *H. braziliensis* fruit shell liquid smoke at different concentrations: 5%, 10%, 25%, 50%, 75%, and 100%. The growth of *S. aureus* bacteria is effectively inhibited by 75% of the *H. braziliensis* fruit shell liquid smoke, with a clear zone measuring 10.6 mm.

Yang *et al.* (2016) evaluated the liquid smoke of *Litchi chinensis*'s MBC and MIC against four different bacterial strains: *P. aeruginosa, A. baumannii, S. aureus*, and *E. coli*. The temperature range for pyrolysis was 100–600°C. The main chemical components of *L. chinensis* liquid smoke, according to GC-MS data, were 2-methoxyphenol (guaiacol) 12.36%; 2,6-dimethoxyphenol (syringol) 29.54%; and 3,5-dimethoxy-4-hydroxytuluene 11.07%. All test bacteria were sensitive to liquid smoke in the disk diffusion method antibacterial test, with inhibition zones ranging from 15 to 19 mm. This study suggests that liquid smoke has a broad antibacterial spectrum against various pathogens, with the maximum inhibition zone (19 mm) against clinical isolates of *S. aureus*, followed by *A. baumannii* and *P. aeruginosa*.

Arundina *et al.* (2020) tested the antibacterial activity of rice husk liquid smoke against *P. gingivalis* ATCC 33277. The highest zone of inhibition was at a concentration of 22.5% (16.72 mm), while the lowest zone of inhibition was at a concentration of 15% (9.98 mm). MIC and MBC were also determined in this study at concentrations of 10% and 12.5%. The antibacterial properties of rice husk liquid smoke created at three different temperatures (300°C, 350°C, and 400°C) was also investigated by Muriady *et al*. (2022). Distillation was the next step used to purify the liquid smoke. According to GC-MS data, phenolic chemicals, butanal, propanoic acid, acetic acid, 2-propanone, and 2-cyclopentene were the main components of rice husk liquid smoke. Tests for antibacterial activity were performed on *B. subtilis, E. coli, S. aureus*, and *S. choleraesuis*. At a pyrolysis temperature of 400°C, liquid smoke produced the best results for suppressing bacterial growth. The diameters of the inhibitory zones that were formed for *S. aureus, B. subtilis, E. coli,* and *S. choleraesuis* were 9.09 mm, 6.69 mm, 7.58 mm, and 7.36 mm, respectively.

Suryani *et al*. (2020) tested the antibacterial activity of eucalyptus (*Melaleuca leucadendra*) and teak (*Tectona grandis*) biomass liquid smoke against *E. coli* FNCC 194,

S. aureus FNCC 0047, and *P. aeruginosa FNCC* 0156. The pyrolysis temperature used in making liquid smoke is 250°C. From the GC-MS results, the main components of the two liquid smokes are known. Eucalyptus biomass liquid smoke contains 45.35% acetic acid and 6.53% phenol while teak wood biomass liquid smoke contains 25.35% acetic acid and 11.59% phenol. From the test results, it is known that the MIC of liquid smoke from *eucalyptus* biomass against *S*. *aureus* FNCC 0047 is 12.5%, while against *P. aeruginosa* FNCC 0156 and *E. coli FNCC* 194 is 6.25%. The MIC of teak wood biomass liquid smoke against *S. aureus* FNCC 0047 was 25%, while that of *P. aeruginosa* FNCC 0156 and *E. coli* FNCC 194 was 3.125%. In 2023, the same researchers tested the antibacterial activity of 2 different liquid smoke, namely sawdust liquid smoke and cocoa pod husk liquid smoke, against 3 strains of bacteria. As a result, the MIC test showed that liquid smoke derived from sawdust biomass (MS) had a lower MIC value than liquid smoke derived from cocoa pod husk (CPH) in all tests against 3 test bacteria. These results confirm that liquid smoke derived from MS has greater antibacterial potential than liquid smoke derived from CPH biomass. The MIC value of liquid smoke derived from MS against *E. coli* FNCC 0091 is 0.39%, while liquid smoke derived from CPH has an MIC value of 3.125% against similar bacteria. The MIC value of liquid smoke derived from MS against *S. aureus* FNCC 0047 was 0.78%, while the MIC value of liquid smoke derived from CPH was 6.25%. The MIC value of MS liquid smoke against *S. typhimurium* FNCC 0150 was 1.56%, while CPH liquid smoke was able to inhibit the growth of *S. typhimurium* FNCC 0150 at a higher concentration of 6.25%.

Desvita *et al.* (2021) also tested the antibacterial activity of cocoa pod shell liquid smoke with pyrolysis temperatures of 300°C (T1), 340°C (T2), and 380°C (T3). *S. choleraesuis* ATCC 14028, *B. subtilis* ATCC 6633*, E. coli* ATCC 25992, and *S. aureus* ATCC 25923 were the bacteria used in the antibacterial activity test. Liquid smoke from cocoa pods has potential antibacterial properties against *E. coli*, *S. choleraesuis, S. aureus,* and *B. subtilis*. Liquid smoke T1 at 5% concentration had the greatest inhibition on *E. coli* and *S. choleraesuis* by 7.17 mm and 7.22 mm respectively, while on *S. aureus,* and *B. subtilis* the greatest inhibition was produced from liquid smoke T3 at 5% concentration. The antibacterial activity of *Castanea sativa* Mill. liquid smoke was evaluated by Misuri & Marri (2021) against 14 bacterial strains that represented both Gram-positive and Gram-negative bacterial species. Liquid smoke was prepared by *pyrogasification reactor* at 75°C. After 24 hours of incubation, both gram-positive and gram-negative bacteria could be inhibited by *Castanea sativa* Mill. liquid smoke with MIC ranging from 0.39% to 1.56%.

According to a number of earlier studies, the lack of lipoprotein and lipopolysaccharide outer membranes makes Gram-positive bacteria more vulnerable than Gram-negative bacteria in general. The outer membranes of lipoproteins and lipopolysaccharides are selectively permeable and have the ability to control the entry of microorganisms into the underlying cell structure (Chan *et al*., 2015). Up to 200 different compounds can be found in liquid smoke's complex chemical composition, which also includes organic acids, pyrans, aldehydes, furans, phenols, and ketones (De Souza Araújo *et al.* 2018). Acetic acid and phenol compounds are essential elements of liquid smoke's antibacterial activities; the presence of organic acids in addition to phenol compounds will enhance their antimicrobial capabilities (Diatmika *et al*., 2019). According to Yang *et al.* (2016) a combination of chemicals works synergistically to produce the antibacterial activity of liquid smoke on bacteria and fungus, rather than a single component acting alone. This fact was emphasized in the study conducted by Suresh *et al*. (2019), who also indicated that each component has a distinct method of

action. Acetic acid is one of the organic acids found in liquid smoke that contributes to the inhibition of bacterial development. By breaking down bacterial cell membranes, acetic acid prevents the growth of bacteria and prevents the creation of enzymes and other macromolecules (Ayudiarti & Sari, 2010). The anionic surfactant characteristics of organic acids cause membrane instability and serve as disinfectants. Organic acids generally work by acidifying the cytoplasm of the cell, which is brought on by the release of extra protons following acid dissociation, to suppress microbial development (Suryani *et al*., 2020). Phenol can act as a poison by inhibiting bacterial enzyme activity and can also denature proteins so that the metabolic activities of bacterial cells die. The process of phenol inactivating proteins is through hydrogen bonds, causing the protein structure to be damaged, which most of the cell wall structure and bacterial cytoplasmic membranes contain fats and proteins (Sadiah *et al*., 2022).

Liquid smoke's antibacterial activity is proportional to its chemical composition, mainly the concentration of phenolic compounds like acids and carbonyls, which are the main factors contributing to its antimicrobial properties; a higher pyrolysis temperature typically results in a greater concentration of these active components, leading to increased antibacterial activity against various bacteria. The type of biomass utilized to produce the smoke also plays a role in its antibacterial activity.

CONCLUSIONS

Liquid smoke has the potential to be a natural antibacterial against both Gram-positive and Gram-negative bacteria, according to the studies mentioned above. The variation in antibacterial sensitivity may result from the concentration of chemical components in liquid smoke, the raw materials utilized for its production, the pyrolysis temperature, discrepancies in test pH, and the methodology employed in testing. The findings of this study can be used by researchers to examine the efficacy of liquid smoke for food preservation, medicine, pesticides, and other applications. Nevertheless, additional research is required to determine the safety of liquid smoke.

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