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Minimum Inhibitory Concentration of Chitosan and Coconut Shell Liquid Smoke Against *Staphylococcus aureus* and *Escherichia coli*

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A B S T R A C T

This study aimed to determine the minimum inhibitory concentration (MIC) of chitosan and coconut shell liquid smoke against *S. aureus* and *E. coli* cultures. The treatments consisted of varying concentrations of Chitosan (0%, 1%, 1.5%, 2%, and 2.5%) and liquid smoke (0%, 1%, 1.5%, 2%, and 2.5%) applied to *S. aureus* and *E. coli* cultures in vitro using nutrient broth medium. The bacterial populations were quantified after 24 hours of incubation at 37°C, with an initial concentration of 10^5 CFU/mL. The results indicated that both chitosan and coconut shell liquid smoke exhibited a stronger bactericidal effect against *S. aureus* than *E. coli*. The minimum inhibitory concentration of Chitosan against *S. aureus* was 1%, resulting in a reduction of 2.16 log cycles, whereas the MIC against *E. coli* was 2%, corresponding to a reduction of 1.47 log cycles. Meanwhile, the minimum inhibitory concentration of coconut shell liquid smoke against *S. aureus* was 1% (reducing 1.542 log cycles), and against *E. coli* was 1.5% (reducing 2.02 log cycles).

Contribution to Sustainable Development Goals (SDGs):
SDG 3: Good Health and Well-Being
SDG 9: Industry, Innovation and Infrastructure

1. INTRODUCTION

1.1. Research Background

Food is a fundamental human need for fulfilling nutritional requirements. An ideal food product should contain sufficient nutrients and energy, possess a long shelf life, be safe for consumption, and remain practical, as these factors influence consumer acceptance. However, food contamination with hazardous chemicals such as formalin and borax—often misused by food producers—remains a serious concern. Monitoring data from the Badan Pengawas Obat dan Makanan (BPOM) evealed that 1.10% of food products failed to meet safety standards out of 9,262 samples tested, with formalin detected in 0.53% of the samples [1]. The widespread circulation of formalin in food products poses a serious threat to consumer health, emphasizing the need for safer preservation alternatives.

The use of appropriate preservatives can extend the shelf life and ensure the safety of food products. Therefore, alternative preservatives derived from natural, non-toxic materials capable of inhibiting microbial growth are highly desirable [2]. *Staphylococcus aureus* and *Escherichia coli* are among the microorganisms that can cause both spoilage and pathogenic contamination in food. *S. aureus* is a Gram-positive, facultative anaerobic coccus that produces enterotoxins and can be found in air, dust, wastewater, milk, food, utensils, and environmental surfaces. *E. coli* is a foodborne bacterium that can cause various gastrointestinal disorders. Although most *E. coli* strains are harmless, some, such as serotype O157:H7, can cause food poisoning in humans [3].

Chitosan and coconut shell liquid smoke have been proposed as promising natural preservatives due to their strong antimicrobial properties. Chitosan, a deacetylated derivative of chitin, is naturally obtained from crustacean shells such as shrimp, crabs, and snails, as well as certain fungi [4]. Liquid smoke, on the other hand, is a condensate produced through the

pyrolysis of lignocellulosic materials containing cellulose, hemicellulose, and lignin [5]. Both compounds contain bioactive components that inhibit a wide range of spoilage microorganisms.

Chitosan is a natural, safe compound that can prolong the shelf life of food due to its bactericidal antimicrobial properties. It disrupts the barrier structure of bacterial cell walls in both Gram-positive and Gram-negative bacteria [6]. Two major compounds in liquid smoke known to exert bactericidal or bacteriostatic effects are phenolic compounds and organic acids [2]. Previous research has shown that *S. aureus* exhibits higher sensitivity to coconut shell liquid smoke compared to *methicillin-resistant S. aureus* (MRSA)[7].

The antibacterial mechanism of Chitosan is attributed to its polycationic structure, which forms an impermeable layer around bacterial cell walls, thereby interfering with the transport of essential substances required for bacterial survival and metabolism [8]. Meanwhile, the antibacterial activity of liquid smoke is primarily associated with its high content of phenolic, carbonyl, and acidic compounds. Coconut shell liquid smoke, in particular, contains phenolic compounds that act as natural preservatives [9]. Therefore, this study aims to determine the minimum inhibitory concentration (MIC) of chitosan and coconut shell liquid smoke against *S. aureus* and *E. coli* cultures in vitro using nutrient broth medium.

1.2. Literature Review

1.2.1. Chitosan

Shrimp shells contain chitin and chitosan compounds, which have high economic value and can be processed for various applications. Chitosan has a wide range of applications and can be utilis in food processing, medicine, biotechnology, and serves as an attractive material for biomedical and pharmaceutical applications [10]. Chitosan is a natural polymer compound derived from chitin, which is obtained from fishery waste such as shrimp and crab shells, containing about 65–70% chitin. Chitosan is a deacetylated product of chitin, produced either through a chemical process using sodium hydroxide (NaOH) or an enzymatic process using chitin deacetylase. The general chemical formula of Chitosan is $C_{18}H_{26}N_2O_{10}$, also known as β-(1,4)-2-Amino-2-Deoxy-β-D-Glucose [11].

The antimicrobial activity of Chitosan is closely related to the protonation of its amino groups. The amino group of Chitosan (NH2) reacts with hydrogen ions (H+) from acetic acid, forming NH3+ groups on the Chitosan. This reaction causes Chitosan to exhibit polyelectrolyte properties. The NH3+ groups interact with the hydrophilic components of the bacterial cell wall, disrupting the activity of cellular enzymes and leading to cell death [12].

The primary mechanism of Chitosan's antibacterial activity involves electrostatic interactions between the polycationic structure of Chitosan and the anionic groups present on the bacterial cell surface. These interactions cause alterations to the cell wall (in Gram-positive bacteria) or the outer membrane (in Gram-negative bacteria), leading to the disruption of cytoplasmic membrane permeability and the subsequent loss of essential cellular constituents,uch as enzymes, nucleotides, and ions, ultimately resulting in bacterial cell death [13].

1.2.2. Coconut Shell Liquid Smoke

Coconut shell liquid smoke is a condensate product derived from the pyrolysis of coconut shells, which produces a large number of simpler compounds formed through the thermal decomposition of constituents such as cellulose, hemicellulose, and lignin. Coconut shell liquid smoke can be applied as a natural food preservative due to its antimicrobial properties, as well as its ability to impart flavor and aroma to food products. The main antimicrobial compounds in coconut shell liquid smoke are phenolic compounds and acetic acid. Phenols exhibit bactericidal and antioxidant properties while also contributing to the flavor of the product [5]. Gas Chromatography–Mass Spectrometry (GC–MS) analysis of liquid smoke has shown the presence of phenolic compounds and acetic acid, both of which possess antimicrobial activity [14].

The mechanism of the antimicrobial activity of phenol and its derivatives involves interactions with the bacterial cell membrane, leading to increased membrane permeability and the leakage of intracellular materials. Additionally, phenolic compounds can inactivate essential enzymes and cause damage or functional inactivation of genetic material. The application of 2% liquid smoke has been reported to inhibit microbial growth in beef jerky compared to samples without liquid smoke treatment [15].

1.2.3. Staphylococcus aureus

Staphylococcus aureus is a Gram-positive bacterium belonging to the family *Micrococcaceae*. The cells of *Staphylococcus* bacteria are spherical in shape and typically arranged in grape-like clusters. The name originates from the Greek words *staphyle*, meaning "a bunch of grapes," and *kokkos*, meaning "berry." *S. aureus* has the ability to produce toxins that can contaminate food. Staphylococcal food poisoning is a foodborne illness caused by the consumption of enterotoxigenic, coagulase-positive *Staphylococcus* strains that produce staphylococcal enterotoxins (SEs) [16].

The optimum temperature for the growth of *S. aureus* ranges from 35 to 37°C, with a minimum growth temperature of 6.7°C and a maximum of 45.5°C. The optimal pH range for *the growth of S. aureus* is between 7.0 and 7.5. Its optimal growth also requires 11 amino acids: valine, leucine, threonine, phenylalanine, tyrosine, cysteine, methionine, lysine, proline, histidine, and arginine [17].

1.2.4. Escherichia coli

Escherichia coli is a Gram-negative, rod-shaped, facultative anaerobic bacterium that can grow and multiply in a wide range of environments and conditions. Several strains of *E. coli* are pathogenic, including *enteropathogenic E. coli* (EPEC), *enteroinvasive E. coli* (EIEC), *enterotoxigenic E. coli* (ETEC), and *enterohemorrhagic E. coli* (EHEC), which can cause various infections in humans and animals such as diarrhea, urinary tract infections, pneumonia, and septicemia. *E. coli* normally inhabits the intestines of humans and animals as part of the gut microbiota. However, certain strains can produce toxins that damage intestinal, renal, or other organ cells, resulting in illnesses such as diarrhea, urinary tract infections, pneumonia, or bloodstream infections [18].

E. coli is commonly used as an indicator organism for fecal contamination. It is part of the normal intestinal flora of humans and animals. *E. coli* can grow within a temperature range of 10–45°C, with an optimal temperature of 37°C. The optimal pH range for its growth is between 7.0 and 7.5, with a minimum pH of 4.0

and a maximum pH of 9.0. The minimum water activity (Aw) value required for *E. coli* growth is 0.96 [19].

1.3. Research Objective

This study aims to determine the minimum inhibitory concentration (MIC) of chitosan and coconut shell liquid smoke against *Staphylococcus aureus* and *Escherichia coli* cultures in vitro in nutrient broth media.

2. MATERIALS AND METHODS

2.1 Material and Tools

The materials used in this study included commercial Chitosan, grade 1 coconut shell liquid smoke, *Staphylococcus aureus* and *Escherichia coli* cultures, Plate Count Agar (PCA), Nutrient Broth (NB), a 0.90% NaCl solution, and a McFarland standard of 0.5. The equipment used consisted of petri dishes, beakers, test tubes, an autoclave, an incubator, a vortex, a Bunsen burner, plastic wrap, analytical scales, a hot plate stirrer, micropipettes, and pipette tips.

2.2 Preparation of Test Cultures

The bacterial test cultures were prepared by taking a loopful of bacteria from a slant of Nutrient Agar (NA) and inoculating it into 10 mL of sterile Nutrient Broth (NB). The suspension was then vortexed to homogenize the bacterial cells and incubated at 37°C for 24 hours. After incubation, the resulting inoculum was used to determine the Minimum Inhibitory Concentration (MIC).

2.3 Determination of the Minimum Inhibitory Concentration (MIC) of Chitosan Against S. aureus and E. coli

A bacterial suspension of 10^5 CFU/mL from a 24-hour NB culture was inoculated into test tubes containing 10 mL of sterile Nutrient Broth. Chitosan was then added to each tube at concentrations of 0%, 1%, 1.5%, 2%, and 2.5%. The tubes were incubated at 37°C for 24 hours. The viable bacterial count of *S. aureus* and *E. coli* was determined using Plate Count Agar (PCA).

2.4 Determination of the Minimum Inhibitory Concentration (MIC) of Coconut Shell Liquid Smoke Against S. aureus and E. coli

A bacterial suspension of 10^5 CFU/mL from a 24-hour NB culture was inoculated into test tubes containing 10 mL of sterile Nutrient Broth. Coconut shell liquid smoke was then added to each tube at concentrations of 0%, 1%, 1.5%, 2%, and 2.5%. The tubes were incubated at 37°C for 24 hours. The number of viable *S. aureus* and *E. coli* cells was determined using Plate Count Agar (PCA).

3. RESULT AND DISCUSSION

The minimum inhibitory concentration (MIC) test of Chitosan against *Staphylococcus aureus* and *Escherichia coli* was conducted in nutrient broth (NB) medium with an initial bacterial population of 10^5 CFU/mL. The growth of *S. aureus* and *E. coli* was observed after 24 hours of incubation.

3.1 Effect of Chitosan Concentration on the Growth of S. aureus and E. coli

The results shown in **Fig. 1** indicate that in the control treatment (0% chitosan), the total of *S. aureus* increased by 1.821 log cycles after 24 hours of incubation (from 5.462 log CFU/mL to 7.283 log CFU/mL). In contrast, treatments with chitosan concentrations ranging from 1% to 2.5% resulted in a decrease in total of *S. aureus* after incubation. At 1% chitosan, the total of *S. aureus* decreased by 2.161 log cycles, while at 1.5% it decreased by 2.792 log cycles. Further increases in chitosan concentration to 2% and 2.5% resulted in a reduction of 3.161 log cycles. Based on these observations of **Fig. 1**, the minimum inhibitory concentration (MIC) of Chitosan against *S. aureus* was determined to be 1%, corresponding to a reduction of 2.161 log cycles after 24 hours of incubation.

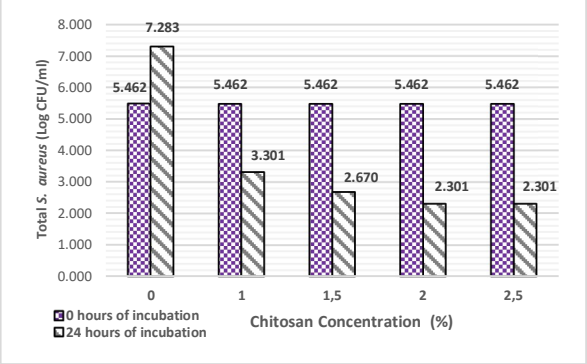

Fig. 1 Effect of chitosan concentration on the total of *S. aureus* in nutrient broth after 24 hours of incubation at 37°C with an initial population of 10^5 CFU/mL.

The results shown in **Fig. 2** indicate that in the control treatment (0% chitosan), the total of *E. coli* increased by 1.325 log cycles after 24 hours of incubation, rising from 5.778 log CFU/mL to 7.103 log CFU/mL. In contrast, treatments with chitosan concentrations ranging from 1% to 2.5% resulted in a decrease in the total *E. coli* count after 24 hours of incubation. At a chitosan concentration of 1%, the total E. coli count decreased by 0.006 log cycles, while at 1.5%, it decreased by 0.112 log cycles. At concentrations of 2% and 2.5%, the total *E. coli* counts decreased by 1.447 and 4.025 log cycles, respectively. Based on the results shown in **Fig. 2**, the minimum inhibitory concentration (MIC) of Chitosan against *E. coli* was determined to be 2%, corresponding to a reduction of 1.447 log cycles after 24 hours of incubation.

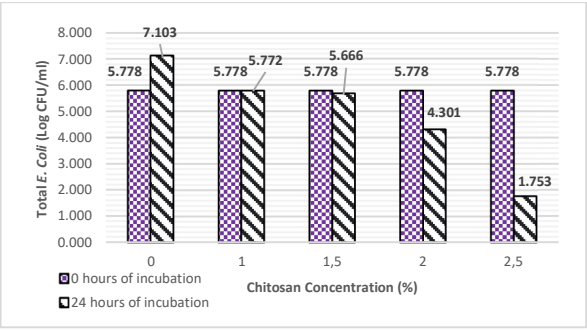

Fig. 2 Effect of chitosan concentration on the total of *E. coli* in nutrient broth after 24 hours of incubation at 37°C with an initial population of 10^5 CFU/mL.

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The antimicrobial mechanism of Chitosan involves electrostatic interactions between the positively charged chitosan molecules and the negatively charged bacterial cell surfaces, resulting in alterations to cell surface permeability. As a result, cellular components such as proteins, amino acids, and glucose leak out, thereby disrupting microbial metabolism and ultimately causing cell death. Additionally, the positive charges of Chitosan can interact with bacterial DNA, resulting inhe inhibition of RNA and protein synthesis [20].

The inhibitory effect of Chitosan was found to be greater against *S. aureus* (a Gram-positive bacterium) than against E. coli (a Gram-negative bacterium). This difference is attributed to variations in cell wall structure between Gram-positive and Gram-negative bacteria. Gram-positive bacteria possess a thick peptidoglycan layer, whereas Gram-negative bacteria have an outer membrane rich in lipopolysaccharides (LPS) [21]. A previous study[22] reported that Gram-positive bacteria are more sensitive to the antimicrobial activity of Chitosan because the outer membrane of Gram-negative bacteria acts as a barrier that limitthe permeability of Chitosan.

Gram-negative bacteria possess three barrier layers—the hydrophobic outer membrane, the peptidoglycan layer, and the cytoplasmic membrane—which collectively restrict the penetration of Chitosan into the cell. In contrast, Gram-positive contain a thick peptidoglycan layer composed of negatively charged teichoic acid molecules. These structural characteristics increase the affinity of Gram-positive toward Chitosan, a cationic polymer, resulting in stronger electrostatic interactions and subsequent cell wall damage [21].

The presence of porin channels in the outer membrane of Gram-negative is also believed to hinder chitosan penetration. Porins are outer membrane proteins that form small channels, allowing passive transport of hydrophilic molecules. They help regulate cellular permeability and contribute to the development of antibiotic resistance. Porins maintain the structural integrity of the Gram-negative membrane and facilitate passive transport of various compounds. Some porins may become more active in the presence of antimicrobial agents, thereby protectingram-negative bacterial cells from inhibition or death [23].

Following a study reported in Ref. [24], it was found that *S. aureus* is more susceptible to Chitosan than *E. coli*. This is due to the presence of teichoic acids on the cell surface of Gram-positive bacteria, which are covalently linked to peptidoglycan, resulting in an overall negatively charged cell surface. Chitosan, being a natural cationic polymer, can bind to these teichoic acids, disrupt their structure, and alter the function of peptidoglycan, thereby enhancing its antibacterial effect.

3.2 Effect of Coconut Shell Liquid Smoke Concentration on the Growth of S. aureus and E. coli

The results presented in **Fig. 3** indicate that treatment with 0% coconut shell liquid smoke led to a 1.821 log cycle increase in the total S. aureus count after 24 hours of incubation (from 5.462 log CFU/ml to 7.283 log CFU/ml). In contrast, at liquid smoke concentrations ranging from 1% to 2.5%, the total of *S. aureus* decreased after 24 hours of incubation. At a concentration of 1%, the total of *S. aureus* decreased by 1.542 log cycles; at 1.5%, the reduction reached 1.779 log cycles; and at 2% and 2.5%, the reductions were 2.342 and 3.102 log cycles, respectively. Based on these observations, the minimum inhibitory concentration (MIC) of coconut shell liquid smoke against *S. aureus* was determined to be 1%, corresponding to a 1.542 log cycle reduction after 24 hours of incubation.

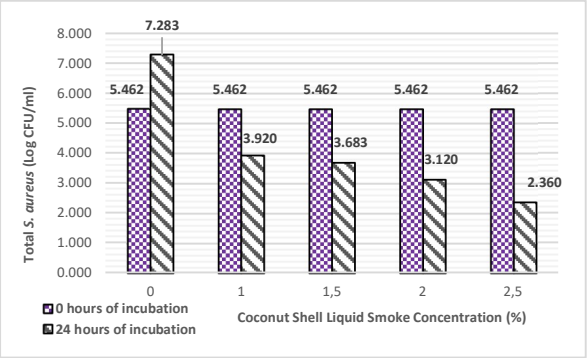

Fig. 3 Effect of coconut shell liquid smoke concentration on the total of *S. aureus* in nutrient broth after 24 hours of incubation at 37°C with an initial population of 10^5 CFU/ml

Observations in **Fig. 4** show that the treatment with 0% coconut shell liquid smoke resulted in an increase in the total of *E. coli* after 24 hours of incubation by 1.325 log cycles (from 5.778 log CFU/ml to 7.103 log CFU/ml). In contrast, treatments with coconut shell liquid smoke concentrations ranging from 1% to 2.5% resulted in a decrease in the total E. coli count after 24 hours of incubation.

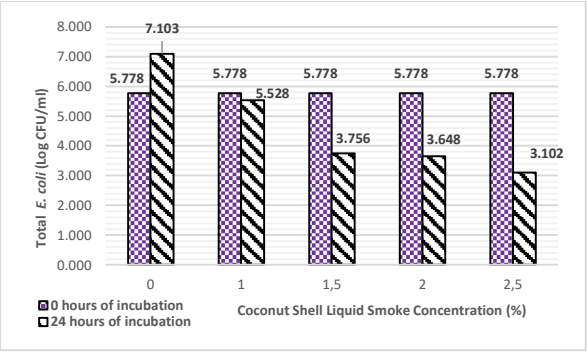

Fig. 4 Effect of coconut shell liquid smoke concentration on the total of *E. coli* in nutrient broth medium after 24 hours of incubation at 37°C with an initial population of 10^5 CFU/ml

At a concentration of 1% coconut shell liquid smoke, the total *E. coli* count decreased by 0.25 log cycles, while at 1.5% concentration, it decreased by 2.022 log cycles. At 2% and 2.5% concentrations, the total *E. coli* count decreased by 2.13 and 2.676 log cycles, respectively. Based on the observations presented in **Fig. 4**, the minimum inhibitory concentration (MIC) of coconut shell liquid smoke against *E. coli* was determined to be 1.5%, corresponding to a reduction of 2.02 log cycles.

Coconut shell liquid smoke contains phenolic compounds that act as antibacterial agents. The mechanism of phenol involves damaging bacterial cell structures and inhibiting cell wall synthesis, leading to cell lysis. The interaction between phenolic compounds and cell membranes also increases membrane permeability, leading to enzyme inactivation and damage to genetic material. The antibacterial compounds in coconut shell liquid smoke penetrate bacterial cells through diffusion. When the concentration of coconut shell liquid smoke outside the cell is higher than that inside, phenolic, acidic, and

carbonyl compounds diffuse into the cell, inhibiting bacterial growth [25].

The inhibitory effect of coconut shell liquid smoke was found to be greater against *S. aureus*. This is likely because *E. coli* can utilise acetic acid as a single-carbon source for survival. Gas Chromatography–Mass Spectrometry (GC–MS) analysis of coconut shell liquid smoke revealed the presence of acetic acid and phenolic compounds [14]. The acetic acid content in coconut shell liquid smoke may decrease as it is metabolized by *E. coli*, thereby reducing its antibacterial activity. This finding is supported by previous studies indicating that *E. coli* can metabolise organic acids (such as acetate) and inorganic salts (such as ammonium sulfate) as sources of carbon and nitrogen[26].

These results are consistent with previous studies reporting that liquid smoke exhibits significantly larger inhibition zones against *S. aureus* compared to *E. coli* [27] [28]. The structural differences between gram-positive and gram-negative bacteria can explain this. *E. coli*, a gram-negative, has a thin peptidoglycan layer surrounded by an outer membrane composed of lipopolysaccharides that act as a barrier to hydrophobic compounds. In contrast, *S. aureus*, a gram-positive, lacks an outer membrane but possesses a thicker peptidoglycan layer. The cell wall of gram-positive bacteria provides greater mechanical and chemical resistance due to its robust peptidoglycan structure, which also increases its susceptibility to disruption by antibacterial agents.

4. CONCLUSION

Both chitosan and coconut shell liquid smoke exhibited stronger bactericidal effects against *S. aureus* compared to *E. coli*. The MIC of Chitosan against *S. aureus* was 1% (resulting in a 2.161 log cycle reduction), while for *E. coli* it was 2% (resulting in a 1.47 log cycle reduction). Meanwhile, the MIC of coconut shell liquid smoke against *S. aureus* was 1% (resulting in a 1.542 log cycle reduction), and against *E. coli* it was 1.5% (resulting in a 2.02 log cycle reduction).

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