



EFFECTIVENESS OF BAMBOO STICK LIQUID SMOKE (*Bamboo* sp.) WITH METHANOL EXTRACT OF SENGON TWIG WOOD (*Falcataria moluccana*) AS A DISINFECTANT

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Abstract

Pathogenic microorganisms that cause infectious diseases are widely spread in the environment, especially in public places that allow microorganisms to multiply. Disinfectants are used to prevent the spread of these microorganisms. The use of chemical-based disinfectants can cause side effects, so it is necessary to look for alternatives from natural materials, such as a mixture of bamboo stem liquid smoke and methanol extract from Sengon twig wood. The purpose of this study was to determine the effectiveness of a mixture of bamboo stem liquid smoke and methanol extract of Sengon twig on bacteria and fungi. This research method began with an inhibition test of the disc and well diffusion method with five comparison treatments (50:50, 40:60, 30:70, 20:80, 10:90) to obtain the most effective comparison of the volume of 100% bamboo stem liquid smoke mixture and 11% sengon twig wood methanol extract, then tested using the Total Plate Count (TPC) method with swab samples on wooden tables. The results showed that the most effective comparison of the volume of 100% bamboo stem liquid smoke mixture and 11% sengon twig wood methanol extract was the volume ratio of 50:50 from the results of the inhibition zone on *Salmonella typhimurium* with a diameter of 10.3 mm. The results of the mixed effectiveness test with a ratio of 50:50 with the swab test on the table showed a decrease in the number of bacteria (51%) and fungi (65%). This shows that the mixture of bamboo stem liquid smoke and methanol extract of Sengon twig wood is effective as a disinfectant.

Keywords: Disinfectant; Bamboo liquid smoke; Sengon extract; Microorganisms

1. Introduction

Microorganisms are widely spread in our environment on walls, tables, chairs, doors, floors and other objects, especially in public places, which allow microorganisms to reproduce.

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Microorganisms are often the cause of infectious diseases, these microorganisms are pathogenic

microorganisms such as bacteria and fungi (Dharmawanti et al, 2021). Bacterial species that cause infections in humans such as *Staphylococcus aureus* and *Salmonella typhi*. Meanwhile, the fungi that often cause infections are *Candida albicans* and *Aspergillus flavus*.

Staphylococcus aureus can cause mastitis infections, dermatitis, or infections of the inhalation tract (Wikananda et al., 2019).

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According to WHO research in 2016, the number of hospital infections in Europe

exceeds 4 - 4.5 million patients every year, and in the United States there is an increase of 1.7 million patients every year. The nosocomial infection rate was 45% of the 99,000 deaths. *Salmonella typhimurium* causes typhoid fever which is generally caused by consuming food or water contaminated with the bacteria. Typhoid fever in Indonesia increases every year, namely by 1.60% in 2018, where the 5 provinces with the largest number are Nanggroe Aceh Darussalam (2.96%), Bengkulu (1.60%), West Java (2.14%) Central Java (1.61%), Banten (2.24%) (Ulfa and Handayani, 2018).

Candida albicans causes candidiasis by infecting the skin, mucosal layers and even internal organs in humans. In women, it is often found in the vaginal area, this can occur due to contamination from nails and water when cleaning themselves or using the toilet simultaneously (Talapko et al., 2021). *Aspergillus flavus* is a fungus that can infect hair, skin, nails, and even cause opportunistic infections. Research conducted at a hospital in Jakarta showed that 7.7% (31 of 405 patients as research subjects) had invasive pulmonary aspergillosis in the ICU. Then research conducted at the Persahabatan National Respiratory Referral Hospital showed results of 68.9% (42 out of 62 patients) in patients after pulmonary tuberculosis infection (Rozaliyani et al., 2019).

Efforts to prevent the spread of pathogenic microorganisms can be done by using liquid disinfectants which are sprayed on surrounding objects with the aim of destroying pathogenic microorganisms (Public Health, 2020). However, continuous use of disinfectants can cause side effects such as dry skin and irritation of children's skin (Dharmawanti et al., 2021). Therefore, we are looking for alternative disinfectants made from natural and environmentally friendly ingredients. The material used is a mixture of sengon twig wood extract with methanol solvent and liquid smoke from bamboo stems.

The extract of sick twig wood from the sengon plant contains secondary metabolites which have the potential for development as natural antimicrobial compounds such as phenolics, flavonoids, saponins, steroids, tannins and terpenoids (Rachmawati et al., 2020). The method used in making the extract is maceration with methanol (polar) solvent with its ability to extract secondary metabolites from the extract (Listiani et al., 2021).

Liquid smoke from bamboo stems is produced from the distillation process, producing cellulose, hemicellulose, lignin, acetic acid and hydrocarbon compounds (Fitriani et al., 2022). The process of making liquid smoke undergoes a pyrolysis process such as at a temperature of 200-250°C it produces acetic acid compounds, a temperature of 300-400°C produces phenol compounds (Pah et al., 2022). Acetic acid compounds have the ability to inhibit spore formation and bacterial growth. And phenolic compounds are effective in in vitro experiments against various organisms such as gram-positive and negative bacteria, yeast and mold (Pah et al, 2022). Therefore, this study aims to determine the effectiveness of a mixture of bamboo stem liquid smoke and methanol extract of Sengon twig wood against bacteria and fungi.

2. Method

This research was conducted at the Microbiology Laboratory of the Rajawali Health Institute in February - May 2023. The research method used in this research was descriptive experimental. Research begins with macroscopic and microscopic identification of microbes.

Then the bacterial and fungal growth curves were measured using a UV-Vis spectrophotometer at a wavelength of 600 nm. Followed by the disk method inhibitory test (Kirby Bauer) for bacteria and wells for fungi, this method was used to determine the volume ratio between bamboo stem liquid smoke (100%) and sengon twig wood methanol extract

(11%) which was the most effective in inhibiting microbial growth from five comparison treatments (50:50, 40:60, 30:70, 20:80, 10:90). Each of them carried out 3 repetitions at each volume ratio of 50:50, 40:60, 30:70, 20:80, and 10:90. A mixture of methanol extract from sengon twig wood and liquid smoke from bamboo stems was dripped 20 microns onto the paper disc and ± 40 microns into the well. Liquid smoke from bamboo stems is obtained by burning it indirectly in an iron drum and the smoke resulting from the combustion will undergo a condensation process and come out through a hole that has been designed as a reservoir for the liquid smoke. After that, the liquid smoke is collected in a bottle and stored at room temperature. The ATCC bacterial culture used was obtained from the West Java Provincial Health Laboratory and ATCC fungal culture from the Faculty of Dentistry, Padjadjaran University.

After obtaining the results of the volume comparison, it is the most effective in inhibiting microbes. Furthermore, a swab test was carried out on the table using the TPC method to determine the effectiveness of the mixture of bamboo stem liquid smoke (100%) and sengon twig wood methanol extract (11%) in reducing the number of microbes. The research began with swab sampling directly on the table after previously the table was given different treatment. The treatment included administering a mixture of 100% bamboo stem liquid smoke and 11% methanol extract of sengon twig wood extract with the best volume ratio, a positive control using a disinfectant liquid made from carbolic acid and a negative control, namely without any treatment.. Each treatment is left for one minute and then swabs are carried out on the table. Furthermore, planting on Nutrient Agar (NA) media to identify the number of bacteria growing, and on Potato Dextrose Agar (PDA) to identify the number of fungi growing. The method used is the pour plate method, with an incubation time of 24 hours on Nutrient Agar (NA) media and 72 hours on Potato Dextrose Agar (PDA).

Some of the tools used in this research are Genesys 10S UV-Vis spectrophotometer, Memmert Germany UN-160 161L oven, All American 24L autoclave, HPX-9272MBE incubator, , Olympus CX23 microscope, laminar air flow, colony counter, petri dish, test tube, cotton swab, and tube. Apart from that, the materials used in this research are: Nutrient Agar (NA), Nutrient Broth (NB), Potato Dextrose Agar (PDA), methanol extract of sengon twig wood with a concentration of 11%, liquid bamboo smoke with a concentration of 100%, solvent methanol, 0.5% H2SO4, 1% BaCl, physiological NaCl, and carbolic disinfectant.

3. Results and Discussion

Mixture Characteristics

In table 1, the characteristics of a mixture of methanol extract from sengon twig wood with a concentration of 11% and liquid bamboo smoke with a concentration of 100% include pH, color and aroma.

Table 1. Mixed characteristics

Concentration	pH	Color	Smell
50:50	3	Orange	Sting
40:60	3	Orange	Sting
30:70	3	Orange	Sting
20:80	3	Orange	Sting
90:10	3	Orange pale	Sting

The methanol extract of sengon twig wood with a concentration of 11% used has the characteristics of a blackish brown color, is semi-solid, and has a pungent odor with a slightly acidic pH. The drying process in making simplicia which will be used as an extract affects the pH produced during the process of increasing the temperature and drying time (Nawawi et al, 2012).

The liquid smoke used in the research was grade 2 bamboo stem liquid smoke with a concentration of 100%. It is bacteriostatic and contains compounds that play an active role as antimicrobials, namely phenolic compounds and acetic acid with an acidic pH of 3.8 (Lestari

et al., 2015). The lower the pH of liquid smoke produces the better quality of liquid smoke which has an effect on inhibiting the growth of microorganisms (Pah et al., 2022).

Identification of Test Microbes

Table 2 shows the results of macroscopic and microscopic identification of test microbes

Table 2. Identification of Test Microbes

No	Types of Microbes	Colony Morphology	Cell Morphology	Staining Results
1	<i>S. aureus</i> ATCC 25923	Round, convex, white	Round in clusters	Gram positive
2	<i>S. typhi</i> ATCC 25241	Round, convex, smooth, white	Bacilli	Gram negative
3	<i>C. albicans</i> ATCC 10231	Round, mucoid, white	Oval-round	Gram positive
4	<i>A. flavus</i> ATCC 9643	Greenery, velvety texture	Round with conidia short	Blue in color

Staphylococcus aureus ATCC 25923 colonies are round, convex elevation, white with gram positive morphology, purple in color, shape *coccus* clustered together. Gram-positive bacteria have thick peptidoglycan so they are able to retain crystal violet dye (Rumidatul et al., 2021). This can occur due to the formation of complex ribonucleate proteins after bleaching (Hamidah et al., 2019).

Salmonella typhimurium ATCC 25241 has round colonies, white color, convex elevation, bacillus-shaped cell morphology, gram negative, red color. In gram staining, when rinsing with alcohol the crystal violet solution will dissolve. Gram-negative bacteria have a thin peptidoglycan layer so that the crystal violet solution cannot bind strongly (Rachmawati, 2020).

Candida albicans ATCC 10231 obtained yellowish white colonies, the colonies on solid medium slightly emerged from the surface of the medium, with a smooth surface, the surface of the colonies was wet and convex, and had a typical yeast smell.

Aspergillus flavus ATCC 9643 has yellow to green colonies, has a velvet-like texture. Results of inoculation on colony PDA medium *A. flavus*, similar to cotton, velvety texture, initial growth of the colony color is white then changes to green with the bottom area of the media yellow.

In the disinfectant, a mixture of 100% bamboo stem liquid smoke and 11% methanol extract of sengon twig wood has antimicrobial content and is able to inhibit the formation of spores and bacteria. So that when the inhibition test is carried out there is an inhibition zone in the media.

Growth Curve

Each test microbe has a different growth rate. The purpose of making a growth curve is to determine the optimum time for the test microbe. The optimum time obtained will be used as a reference for conducting effectiveness tests. The results of the growth curve test are presented in Figure 1.

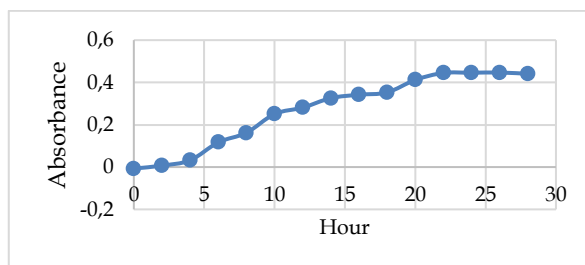


Figure 1. Growth Curve Graph *S. aureus* ATCC 25923

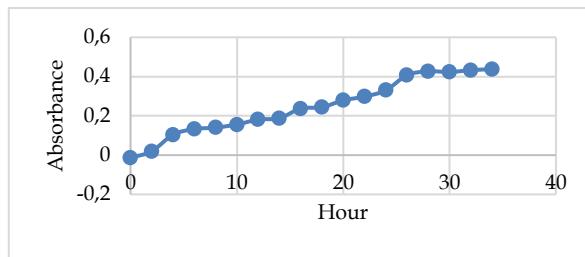


Figure 2. Growth Curve Graph *S. typhimurium* ATCC 25241

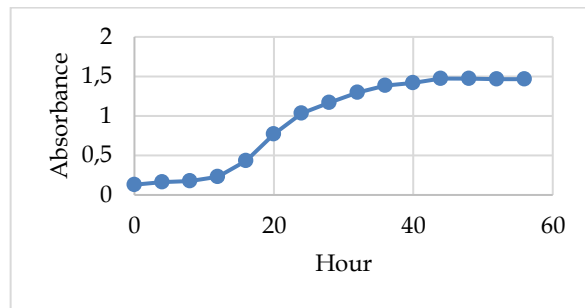


Figure 3. Growth Curve Graph *C. albicans* ATCC 10231

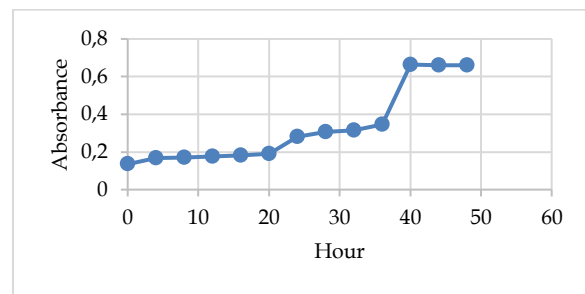


Figure 4. Growth Curve Graph *A. flavus* ATCC 9643

Optimum phase *S. aureus* at the 22 hours, the optimum phase *S. typhimurium* at the 30 hours, the optimum phase *C. albicans* at 44 hours, and the optimum phase *A. flavus* at 40 hours. Making a growth curve by measuring the absorbance of colonies in liquid media using a UV-Vis spectrophotometer using a wavelength of 600 nm. The wavelength used is the optimal wavelength for measuring turbidity from yellow to brown (Listiani et al., 2021).

There are four phases in the microbial growth rate, namely the lag phase, log phase, stationary phase and death phase. In the lag phase when the test microbes are inoculated into the media, the test microbes are still adapting to the environment. In the lag phase, no additional cells occur, indicated by the color of the liquid medium which is not yet cloudy. The log phase is the phase when actively growing microbes carry out rapid cell division. In the log phase, the addition of cells begins to occur because the number of cells increases, indicated by the liquid media starting to become cloudy, where the test microbes begin to adapt and consume the nutrients contained in the media. The stationary phase, namely the cell growth phase, occurs in a balanced manner because the number of cells that die is the same as the number of cells that grow. In the stationary phase, the number of cells increases is equal to the number of cells that die because the nutrients in the medium decrease and a struggle for nutrients begins to occur. The final phase, the death phase, occurs because cell growth stops because the nutrients in the media run out so that the number of microbial cells that die is higher (Padoli, 2016). In this study, the growth curve test did not reach the death phase, because it was only to determine the optimum time for collecting colonies for the inhibition test.

Antimicrobial Inhibition Test

The inhibition test findings show that *S. aureus* may be effectively suppressed by 5.6 mm at a volume ratio of 10:90 between Sengon twig wood methanol extract and bamboo stem liquid smoke. The outcomes are shown in Figures 2 and 3 below. Inhibition zone area *S. typhimurium* 10,3 mm at a ratio of 50:50. Inhibition zone area *C. albicans* 2,5 mm at a volume ratio of 50:50. Meanwhile, the area of the inhibition zone at *A. flavus* 4,17 mm at a volume ratio of 40:60.

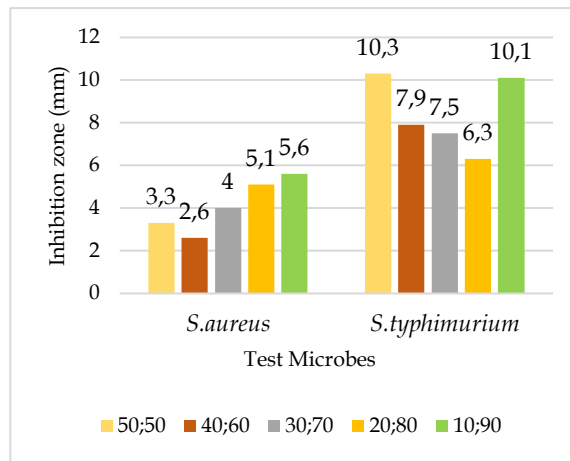


Figure 2. Bacterial Inhibition Test Result

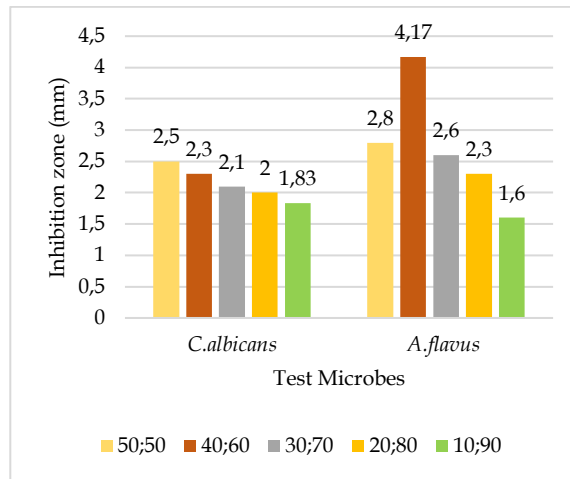


Figure 3. Fungal Inhibition Test Result

Based on the results of test research *S. aureus* are gram-positive bacteria which are composed of a thicker peptidoglycan layer compared to *S. typhimurium* which is a gram-negative bacterium that has a thinner peptidoglycan layer than *S. aureus* (Hayati et al., 2020).

Candida albicans is a fungus that has true hyphae which function as defense so that it is difficult to penetrate (Chairini and Harfiani, 2018). Meanwhile, *Aspergillus flavus* It has 10-30% more chitin which functions as a constituent of peptidoglycan as a component of cell walls for self-defense (Hasibuan et al., 2021).

Swab Test on the Table

In Figure 4, it can be seen that the average results of the percentage reduction of microbial count show that the effectiveness of a mixture of bamboo stem liquid smoke and methanol extract of Sengon twig wood on bacterial growth is 51% and fungi is 65%.

Carbol is an indicator of the percentage of the disinfectant's inhibition zone on the growth of the tested microbes.

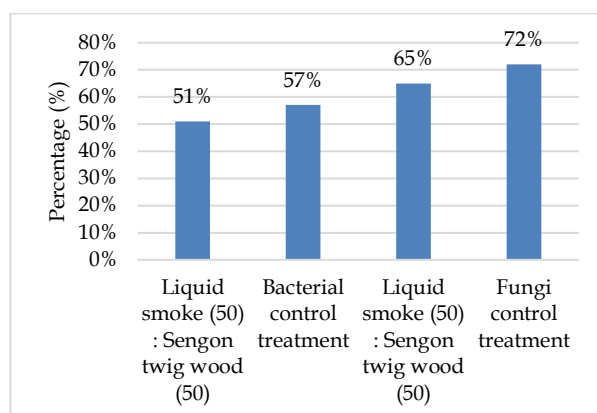


Figure 4. Effectiveness Reducing the Amount of Microbial Growth

This inhibitory ability is influenced by the contents of bamboo stem liquid smoke and sengon twig wood extract using methanol solvent. According to Pah et al., (2022) Compounds that are often used as antibacterials in liquid smoke are phenol, carbonyl and acid compounds. Phenolic compounds can kill bacterial cells by denaturing bacterial cell proteins. At low concentrations, phenol compounds can damage the cytoplasmic membrane and cause leakage of the cell nucleus. At high concentrations, phenolic compounds coagulate with cellular proteins (Kristiana et al., 2023). According to Ridolf et al., (2018), carbonyl has an important role in the color and taste produced from liquid smoke.

According to Rumidatul et al (2018), Sengon plants have phytochemical compounds called secondary metabolite compounds. This secondary metabolite compound is thought to have antimicrobial, antioxidant and anthelmintic properties. Sengon twig wood extract using methanol as a solvent contains a variety of secondary metabolite compounds. These secondary metabolite compounds are terpenoids, steroids, flavonoids, phenolics, tannins and saponins.

The mechanism of action of terpenoid compounds as antibacterial is thought to involve membrane damage by lipophilic compounds. Furthermore, steroid compounds are known to have cardiotoxic and antibacterial activity. Steroids act as antibacterial by damaging bacterial cell membranes (Rumidatul et al., 2020). According to Waluyo (2016), flavonoids can kill bacteria directly and reduce bacterial pathogenicity. In addition, flavonoids are active compounds that have antibacterial properties because they can form complexes with extracellular proteins and soluble proteins with bacterial cell walls. Phenolic compounds at low concentrations have the ability to damage the cytoplasmic membrane and cause leakage of the bacterial cell nucleus. In contrast, high concentrations of phenolic compounds bind to cellular proteins (Haryati et al., 2015).

Tannin's ability as an antibacterial can be seen from its action on membranes. Due to the ability of tannins to precipitate on proteins, they can pass through cell membranes. Tannins have antifungal properties in addition to being antibacterial. Has the ability to prevent chitin synthesis, which leads to the formation of cell walls in fungi. This damages cell membranes and inhibits fungal growth (Kurniawati et al., 2016). Lastly, there is the saponin compound, this compound can inhibit the growth of bacteria. Saponins inhibit bacterial growth by reducing the ability of microorganisms to utilize glucose, affecting growth and proliferation, reducing the activity of important enzymes in physiological metabolism, and suppressing protein synthesis, which in turn causes cell death (Nath et al., 2022). Apart from inhibiting the growth of bacteria, saponin compounds can also inhibit the growth of fungi. In fungi, Saponin works by disrupting the integrity of *C. albicans* cells (Kurniawati et al., 2016).

4. Conclusions and suggestions

Based on the research that has been carried out, it can be concluded that the mixture of bamboo stem liquid smoke and methanol extract of sengon twig wood has antimicrobial activity against *Staphylococcus aureus* ATCC 25923, *Salmonella typhimurium* ATCC 25241, *Candida albicans* ATCC 10231, and *Aspergillus flavus* ATCC 9643. This shows that the mixture of bamboo stem liquid smoke and methanol extract of sengon twig wood is effective as a disinfectant raw material because it can inhibit the growth of bacteria and fungi.

Further research is needed to identify the compounds contained in the mixture of bamboo stem liquid smoke with a methanol extract of Sengon twig wood.

5. Thank You

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