JURNAL BIOMEDIKA
Volume 15 No. 02 September 2022
P-ISSN: 1979 - 035X (printed) & E-ISSN: 2302 – 1306 (online)
DOI: https://doi.org/10.31001/biomedika.v15i2.1543

Effectiveness of Antiseptic Liquid Smoke of Pine Fruit (*Pinus merkusii*) in Vitro and in Vivo

Fhadliana Alfani[1], Diana Rizki Armela[1], Fa'izzah Nur Adzra[1], Feldha Fadhila[1], Yayan Maryana[1], Alfi Rumidatul[2*]

[1]Fakultas Kesehatan, Instititut Kesehatan Rajawali, Bandung, Jawa Barat, 40184, Indonesia
[2]Sekolah Ilmu dan Teknologi Hayati, Institut Teknologi Bandung, Bandung, Jawa Barat, 40116, Indonesia

ARTICLE INFO

Article History:
Received: November, 2021
Revise: November 2022
Accepted: December 2022

ABSTRACT

Hand sanitizers containing alcohol-based components are frequently used to clean hands due to their usage is more effective and efficient. Natural substances, such as pine fruit, can be used to replace alcohol-based components. Pine fruit may be processed into an antibacterial liquid smoke product, making it potentially useful as an antiseptic. The purpose of this research is to see how effective pine fruit liquid smoke is at inhibiting microbial growth in vitro and in vivo. In vitro disc diffusion test against *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923, and employing disc and well diffusion methods on *Aspergillus flavus* ATCC 9643 and *Candida albicans* ATCC 10231. The pour plate approach was used in vivo. The in vitro test findings revealed an inhibitory zone on *E. coli* (5.3 mm) and *S. aureus* (5.83 mm) from 100% concentration of liquid smoke. The in vitro test results are supported by the in vivo test results, which indicate that the liquid smoke of pine fruit is 100% effective at preventing the growth of bacteria by 75.1% and fungi by 87.7%. The results of the questionnaire indicated that respondents liked the color (50%), and aroma (58%), that the product did not induce dryness (75%), and did not burning effects (83%). The result of this research is that 100% liquid smoke of pine fruit (*Pinus merkusii*) is effective in inhibiting the growth of *E. coli* ATCC 25922 and *S. aureus* ATCC 25923, as well as being effective as antiseptic agents.

Keywords:
antiseptic; liquid smoke; pine fruit, *Pinus merkusii*

*Corresponding authors:
Alfi Rumidatul
School of Life Sciences and Technology, Bandung Institute of Technology, Bandung, West Java, Indonesia
Email: alfi@sith.itb.ac.id

INTRODUCTION

Diseases can be transmitted through contaminated food and water, as well as from person to person as a result of inadequate hygiene. In general, the digestive system is manifested by *Escherichia coli* (*E. coli*) as diarrhea (Hutasoit, 2020). In 2017, there were 1.7 billion diarrhea cases, with a mortality rate of approximately 525,000 in children under the age of five (WHO, 2017). In 2016, the prevalence of nosocomial infections in Europe was predicted to be between 4 and 4.5 million people yearly, whereas in the United States it was anticipated to be approximately 1.7 million patients. This prevalence accounts for 4.5 % of 99,000 deaths. *Staphlococcus aureus* (*S. aureus*) is responsible for nosocomial infections (WHO, 2016).

Candida albicans (*C. albicans*) is one of the infectious disorders that can be caused by fungi which organism causes candidiasis (Pulungan, 2017). *Candida albicans* is an opportunistic fungus infection that can infect individuals with a weakened immune system, including those with diabetes, HIV, leukemia, or who are pregnant (Makhfirah et al., 2020). Human instaces of candidiasis infection have been documented in 64.8% of cases and are growing annually (Kalista, 2017). In addition, there is a fungus *Aspergillus flavus* (*A. flavus*), that produces mycotoxins because it contaminates food. This fungus causes lung infections (Hasanah, 2017).

By washing hands and applying an antiseptic, you can prevent certain microbiological infectious diseases. Alcohol-based antiseptics are believed to be capable of inhibiting bacterial growth and damaging bacterial cell membranes (Kusuma et al., 2019). However, the continued use of alcohol-based antiseptics can cause a scarcity of alcohol as a result of its rising consumption (Asngad et al., 2018).

The liquid smoke of pine fruit has antibacterial properties and is one of the natural compounds being considered as an alcohol alternative. Pine trees have active substances in resin channels that are located not just in the trunk, but also in nearly every other part of the plant, including the fruit, roots, twigs, and leaves (Senjaya & Surakusumah, 2007). According to Aisyah et al. (2018), pine fruit liquid smoke can prevent *Ralstonia syzygii* subsp. *celebesensis*, the cause of blood disease in banana plants in Indonesia. Due to the presence of acid, phenol, and alcohol components in liquid smoke, which are known to hinder bacterial development, its usage as a hand antiseptic can have an impact on bacterial growth (Fauziati, 2012).

Based on this, a research was conducted on using pine fruit liquid smoke both in vitro and in vivo as an antiseptic. *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *A. flavus* ATCC 9643, and *C. albicans* ATCC. 10231 were among the test microbes used in an in vitro research to determine the efficiency of pine fruit liquid smoke at concentrations of 50%, 75%, and 100% in preventing the growth of the test microbes. The respondent's palms were then subjected to an in vivo follow-up test using pine fruit liquid smoke. The in vivo test was used as an antiseptic to back up the in vitro efficacy test results. The purpose of this research is to establish the optimal concentration of pine fruit liquid smoke (50%, 75%, and 100%) that is useful as an antiseptic in suppressing the growth of germs and fungi.

MATERIALS AND METHODS

Types of Research, Place and Time

The research method used is experimental research. In March 2021, this research was carried out in the Laboratory of Microbiology, Faculty of Health, Rajawali Health Institute

Population, Samples and Sampling Techniques

This research was carried out both in vitro and in vivo. In the in vitro study, the population consisted of bacterial isolates from the West Java Provincial Health Laboratory and fungal isolates from the Faculty of Medicine, University of Padjadjaran. The sample used is suspension *E. coli* ATCC 25922 and *S. aureus* ATCC 25923, *A. flavus* ATCC 9643 and *C. albicans* ATCC 10231.

The general public is the population studied in this in vivo research. The sample size was 12 respondents (6 for the liquid smoke treatment, and 6 for the 70% alcohol treatment). Random sampling was used in this research.

Materials and Tools

The materials employed were grade 3 pine fruit liquid smoke (50%, 70%, and 100% concentrations), test microbiological suspension samples, 70% alcohol, 0.9% NaCl, palm swab samples, and distilled water. The following growth mediums were used in this research are Nutrient broth (NB), Nutrient Agar (NA), dan Potato Dextrose Agar (PDA). The tools used in this research were a xingweiqiang type pH meter, Genesys 10S UV-Vis spectrophotometer, HPX-9272MBE incubator, All American 24L autoclave, Memmert Germany UN-160 161L oven, Olympus CX23 microscope, petri dish, test tube, cotton swab, volume pipette, drip pipette, beaker, round ose, and spirtus lamp.

Research Steps

This research started with an in vitro test to identify the test microbes, which uses gram staining on bacteria and yeast and methylene blue staining on molds.

A UV-Vis spectrophotometer with a wavelength of 600 nm was also used to measure the growth curve of the test microbiological solution. The zone of inhibition was next examined on bacteria using the disc diffusion method (*Kirby Bauer*) and wells, and on fungi using the disc diffusion method (*Kirby Bauer*) and wells. Each test method was treated three times with pine fruit liquid smoke at concentrations of 50%, 75%, and 100% as much as 20 µl disc paper and ± 25 µl for wells.

The pour plate method was used for the in vivo test. Based on the results of the optimal concentration in vitro, liquid smoke pine fruit was utilized. The therapy consisted of applying 2 mL of pine fruit liquid smoke to the respondent's palm (treatment), followed by 70% alcohol (positive control). The microbiological count was then performed using the Total Plate Count (TPC).

Secondary data was acquired by a questionnaire, with the factors employed including color preference, scent preference, the effect of dryness on the skin, and the effect of itching and burning on the skin. The quiz utilizes a scale of 0 for "dislike," 1 for "normal," 2 for "like," and 3 for "very like." Respondents who have not cleansed their hands with soap or hand sanitizer, do not have antiseptic allergies, and do not have wounds on their palms were chosen as the sample criterion.

Data Analysis

The descriptive analysis is utilized to analyze the data for this research. The data is presented in tabular form and narratively explained.

RESULTS AND DISCUSSION

Characteristics of Pine Fruit Liquid Smoke

The results of the research on the properties of the liquid smoke of pine fruit, it has a blackish brown color, is translucent, has a strong aroma, and has a pH that fluctuates with concentration, specifically 4.9, 4.5, and 3.7 at 50%, 75%, and 100% concentrations (Table 1).

The presence of tar components influences the color of liquid smoke, whereas the presence of phenolic compounds influences its pungent scent. The presence of organic acid chemicals, phenols, and alcohol contribute to the acidic quality of liquid smoke. The pH value of liquid smoke can affect the quality of liquid smoke; the lower the pH value, the higher the quality of liquid smoke. As a result, the pH value can impact liquid smoke's ability to prevent microbial development (Aisyah, 2019).

It contains bioactive chemicals, such as phenol, alcohol, and acids, liquid smoke is widely utilized as an antibacterial. The amounts of wood components, notably lignin, cellulose, and hemicellulose, affect the concentration of these bioactive chemicals. The higher the lignin concentration, the higher the phenol content in the liquid smoke, whereas the high amounts of cellulose and hemicellulose wood components influence the high levels of alcohol and acid compounds in the liquid smoke (Aisyah, 2019).

Table 1. Characteristics of Pine Fruit Liquid Smoke

No.	Physical Properties	Pine fruit liquid smoke 50%	Pine fruit liquid smoke 75%	Pine fruit liquid smoke 100%
1	Color	Blackish brown	Blackish brown	Blackish brown
2	Transparency	Transparent	Transparent	Transparent
3	Aroma	Sting	Sting	Sting
4	pH	4,9	4,5	3,7

Microbial Identification Test

Macroscopic examinations identified *E. coli* ATCC 25922 as having round colonies, a milky white hue, convex elevation, and flat edges. *Eschericia coli* ATCC 25922 has the shape of rods or monobacilli, is red in color, and is gram negative (Table 2). Gram-negative bacteria have a thin peptidoglycan layer and a thick lipid layer. During the decolorization process, the lipid layer is degraded by alcohol and loses its ability to maintain the primary color of crystal violet. As a result, the lipid layer absorbs the second dye, safranin, which gives bacterial cells their red color (Muharini et al., 2017).

Upon macroscopic examination of *S. aureus* ATCC 25923 colonies, it was determined that they are round, white, have convex elevation, and flat edges. Gram staining revealed *S. aureus* ATCC 25923 to be round or cocci, clustered like grapes, purple in color, and gram positive (Table 2). Gram-positive bacteria have a cell wall structure with a higher peptidoglycan concentration, therefore they retain crystal violet dye, which is purple (Purwaningsih & Wulandari, 2021).

Candida albicans ATCC 10231 colonies were observed to be oval-shaped, yellowish-white, to have convex elevation, smooth and flat edges, and to have a yeasty odor. Using gram staining, the *C. albicans* ATCC 10231 cell morphology was determined to be oval, to contain blastospores and peudohyphae, and to be gram-positive. *Aspergillus flavus* ATCC 9643 colonies were seen to be granular, with velvet-like granules and a greenish-yellow hue upon macroscopic examination. *Aspergillus flavus* ATCC 9643 was stained microscopically with methylene blue, and its cell morphology consisted of conidides, transparent conodiophores, and spherical fesicles (Table 2).

Figure 1(a) demonstrates that the *E. coli* optimal phase occurred at 18 hours (b). Figure 1(a) depicts the optimum phase of *S. aureus* at the 30th hour, Figure 1(c) depicts the optimum phase of *C. albicans* at the 20th hour, and Figure 1(d) depicts the optimum phase of *A. flavus* at the 15th hour. In general, the growth curve goes through four phases: adaptation phase (lag), exponential phase (log), stationary phase, and death phase (Sharah et al., 2015). The lag phase is characterized by a rise in the number of microorganisms as they acclimatize to ambient circumstances governed by pH, temperature, and nutrition (Mardalena, 2016). Furthermore, the log or exponential phase characterizes a cell that divides at a constant pace, doubles its mass at the same rate, has constant metabolic activity, and is in a state of balanced growth (Risna et al., 2022). The medium in which it grows, such as pH and nutrient content, as well as ambient factors such as temperature and humidity, all have a substantial influence on the rate of growth during this period (Setyati et al., 2015). The following phase is the stationary phase, which occurs when the number of cell deaths equals the number of cell growth. Many secondary metabolites are produced during this phase because bacteria defend themselves to survive by producing secondary metabolites, and some are poisoned by changing environmental conditions as a result of the metabolites produced (Bertrand, 2019). The death phase occurs during the 20th and subsequent hours, when the number of dead cells exceeds the number of living cells and the medium runs out of nutrients, causing the bacterial population to decline in size (Mahjani & Putri, 2020).

Table 2. Macroscopic and Microscopic Identification of Test Microbes

No.	Microbial Test	Characteristic Colony	Cell Characteristics	Result Coloring
1	*Escherichia coli* ATCC 25922	Round, milky white, convex, flat edges	Stem, monobasil, red	Gram negative
2	*Staphylococcus aureus* ATCC 25923	Round, white, convex, flat edges	Round, purple, clustered like grapes	Gram positive
3	*Candida albicans* ATCC 10231	Round, yellowish-white, convex, flat edges	Round, purple, there is psedohifa	Gram positive
4	*Aspergillus flavus* ATCC 9643	Semi-rounded, greenish-yellow, no elevation, uneven edges	Round, blue, there's a conidia	Blue Color

Microbial Growth Curve

The growth curve is used to determine the optimal growth phase of the test microorganisms, after which the measurement of the growth curve shown in Figure 1 is performed.

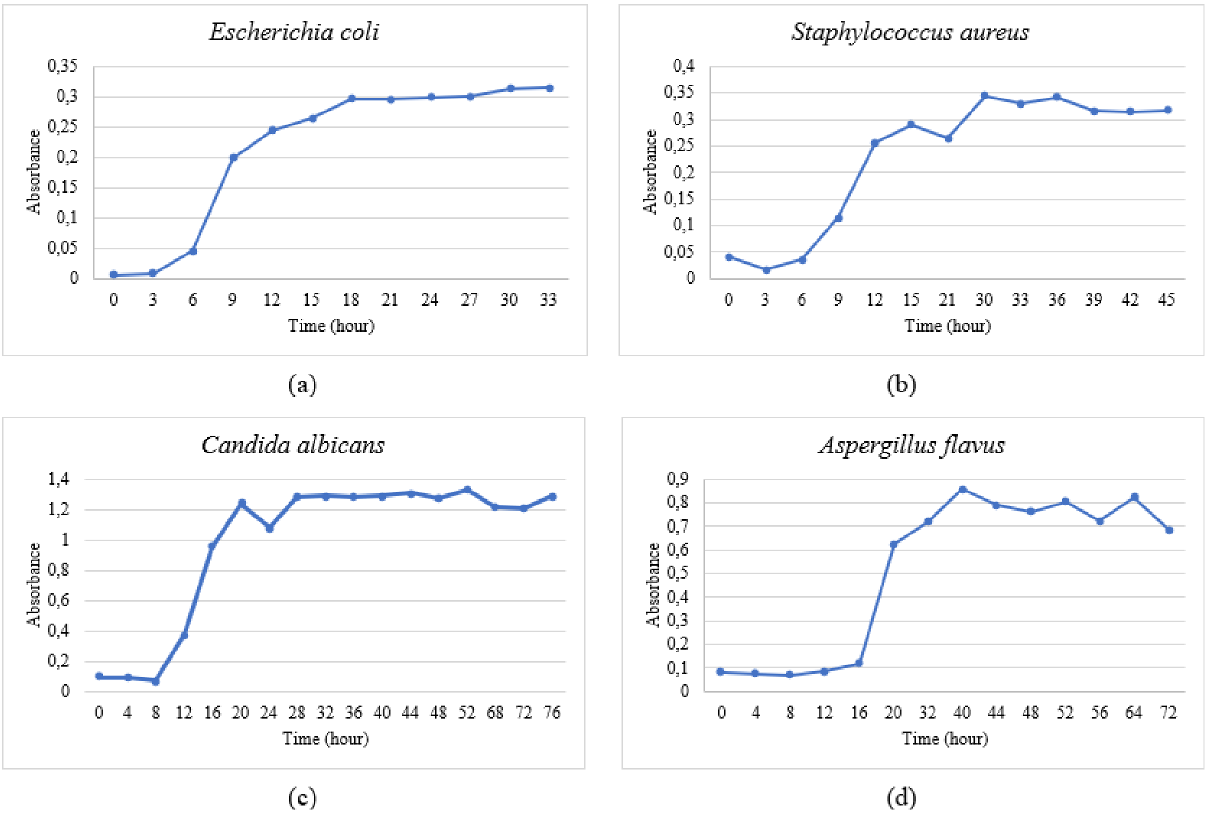

Figure 1. Growth Curve of Test Microbes

Antimicrobial Inhibition Zone Test

Figure 2 depicts the inhibition zone test, which demonstrates that the biggest inhibition zone created in *E. coli* is 5.3 mm and in *S. aureus* is 5.8 mm when the optimum concentration of pine fruit liquid smoke is 100%. Meanwhile, at varying concentrations of pine fruit liquid smoke, *C. albicans* and *A. flavus* did not develop inhibitory zones. In the control, each test microorganism had a separate inhibition zone, however in the negative control, no inhibition zone was generated (Figure 2).

The test bacteria established an inhibitory zone with the optimum concentration of antimicrobial liquid smoke of pine fruit, precisely 100%, according to the findings of the test. Previous research has found that the higher the concentration of liquid smoke, the lower the pH value or the more acidic the environment. The presence of large quantities of organic acids and phenols, which function as antimicrobials, contributes to the low pH value (Pudja et al., 2020). Liquid smoke contains acid and phenolic chemicals that can penetrate and destroy bacterial cell walls as well as precipitate proteins in bacterial cells. Furthermore, phenol can promote protein coagulation, alter the permeability of bacterial membranes, and eventually cause cell membrane lysis (Aisyah, 2019).

The inhibitory zone test results on *S. aureus* ATCC 25923 were higher than those on *E. coli* ATCC 25922. Gram-positive bacteria have a basic cell wall structure with more peptidoglycan, whereas gram-negative bacteria have a complicated cell wall structure with little peptidoglycan and a lot of lipoposaccharides, which are carbohydrates that are attached to lipids (Purwaningsih & Wulandari, 2021).

Figure 2 shows the findings of the observations on the test mushrooms, namely *C. albicans* ATCC 10231 and *A. flavus* ATCC 9643, which did not produce an inhibitory zone in the disc or well diffusion method. The growth of *C. albicans* ATCC 10231 and *A. flavus* ATCC 9643 was not inhibited by pine fruit liquid smoke at varied doses. These findings contradict prior research, which found that sawdust liquid smoke at a concentration of 2% inhibited the growth of *A. flavus* (Aisyah, 2019). *Candida albicans* growth can be inhibited by liquid smoke produced by bamboo pyrolysis (Dediwanto et al., 2020). Bioactive components in liquid smoke, such as alcohol, phenol, and acid, are germicidal because they can prevent mold spore germination and limit hyphae growth (Aisyah, 2019). However, some fungi, such as fungus with mycelium, have components that can inhibit an antibiotic, and these fungi can be antibiotic resistant (Lan et al., 2018). Fungi are classified into two groups: yeasts and molds. Molds have cell walls that contain 10%-30% chitin compounds, whereas yeasts have cell walls that contain 0.6%-9% chitin compounds (Agnol et al., 2003). The chitin compound contributes to the production of cell walls in fungi, and there is extra content in the yeast group, specifically the ergosterol component, which is a component of the plasma membrane and contributes to the formation of chitin compounds in the fungal cell wall of the yeast group. As a result, it will not be effective in preventing the growth of *A. flavus* and *C. albcans* apart from antifungals (Bowman et al., 2006).

In Vivo Test Results

According to the percentage reduction in the number of microbes, the efficiency of pine fruit liquid smoke on bacterial development was 75.1% on average, and 87.3% on average for fungus. Alcohol can be used to calculate the percentage of antiseptic inhibition on microbiological growth (Figure 3).

The percentage of the efficiency of liquid smoke as an antiseptic was calculated using an in vivo pine fruit liquid smoke effectiveness test to reduce the number of bacterial and fungus colonies. The in vivo test was used as an antiseptic, and the results of the in vitro effectiveness test were supported. The responders' palms were treated with 100% concentrated pine fruit liquid smoke. In this study, there were 12 samples that were separated into two groups of respondents, group X (swiping the palms before and after using 100% pine fruit liquid smoke) and group Y (swiping the palms before and after using alcohol 70%), with each group consisting of 6 respondents. The proportion of women to men is equal in each group.

The outcomes of the in vivo tests (Figure 3) demonstrate the ability of 100% pine fruit liquid smoke to prevent the growth of bacteria and fungi. Aisyah (2019) asserts that liquid smoke has the capacity to prevent bacterial growth. Liquid smoke possesses fungistatic qualities, which prevent the growth and extension of fungal hyphae, in addition to germicidal capabilities that can interfere with the production of fungal spores. The alcohol, acid, and phenol groups found in liquid smoke are bioactive substances that have the power to collectively impede the growth of microbes because they interfere with their physiological structure.

Compared to 70% alcohol, 100% pine fruit liquid smoke has a lesser efficacy value. It is believed that the liquid smoke contains contaminants. Grade 3 liquid smoke, according to Aisyah (2019), still has a lot of tar in it, giving it the physical characteristics of being black in color and having deposits.

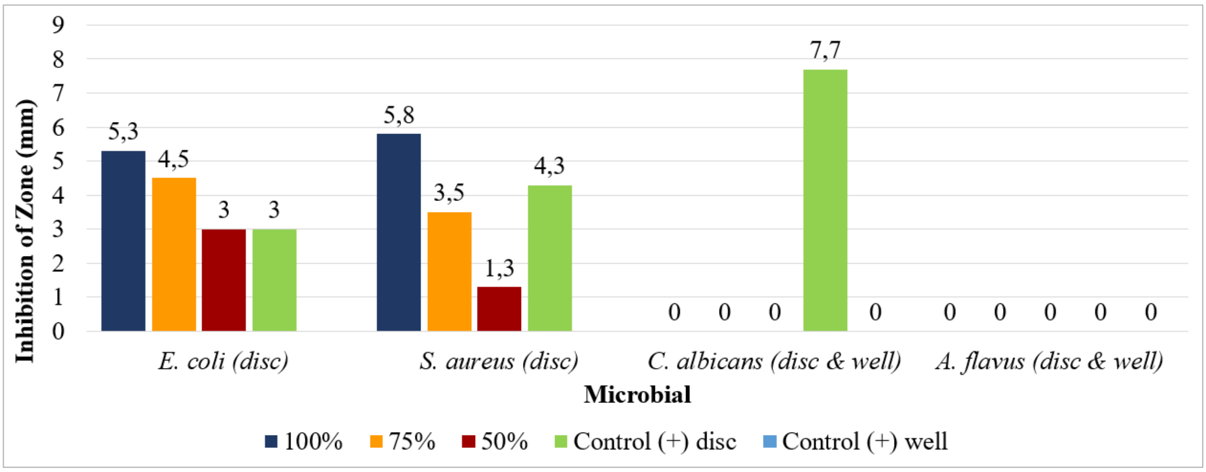

Figure 2. Zone of Inhibition of Pine Fruit Liquid Smoke against Test Microbes

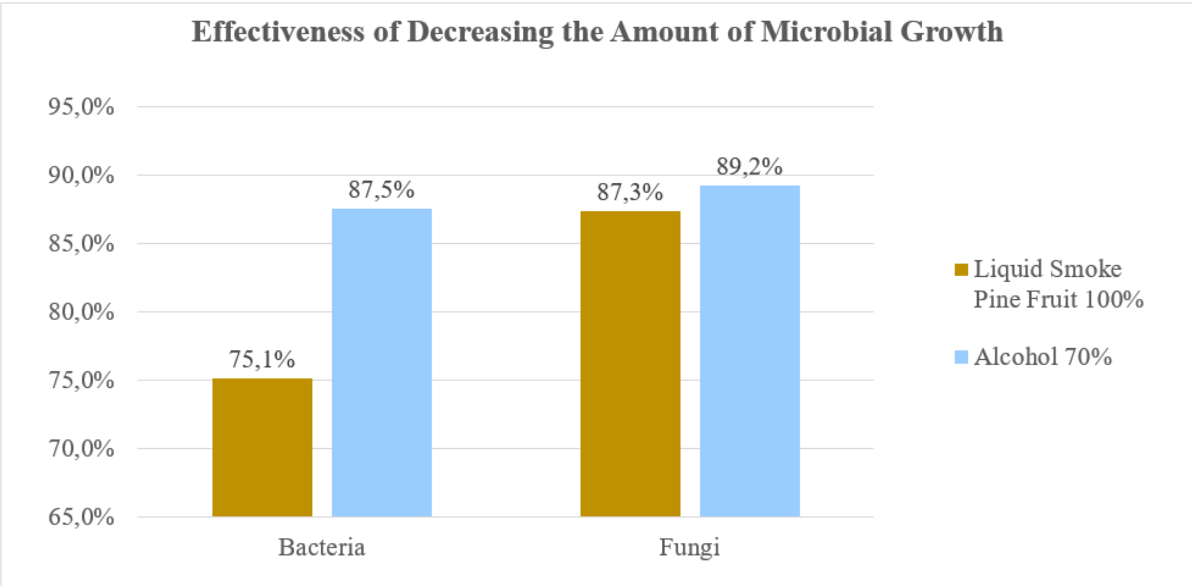

Figure 3. Effectiveness of Decreasing the Amount of Microbial Growth

Questionnaire Results

This research included a questionnaire to determine the viability of pine fruit liquid smoke as an antimicrobial. In Figure 4, it can be observed that respondents enjoyed the color of pine fruit liquid smoke (50%), the scent of pine fruit liquid smoke (58.3%), the fact that liquid smoke goods did not dry out the skin (75%), and that smoke liquid did not create a burning feeling (83.3%). Some responders (50%) did not appreciate the pine fruit liquid smoke's dark tint, which is described as blackish brown. The highest level of preference for the color of hand sanitizer, according to research by Aznury et al. (2020), is green because the color is lighter and more appealing, whereas the dark hue is less appealing to the public. The choice of color for a product is crucial to its ability to attract a large number of people. A small percentage of respondents (41.7%), due to its pungent aroma, did not like the smell of pine fruit liquid smoke. This was consistent with previous studies, which found that the level of public preference for the aroma was higher in hand sanitizers that were not too strong, and that people did not like hand sanitizers with a very pungent smell. Some respondents (25%) disliked pine fruit liquid smoke due to its drying effect on the skin; in addition, 16.7% of respondents had a burning or hot sensation. Therefore, people are more comfortable using products that feel cool and non-sticky while not in use, and they prefer hand sanitizers with a gel texture as opposed to a liquid texture. The use of hand antiseptic goods or hand sanitizer is sticky, feels hot, and irritates the skin, so compromising the health of the user. Therefore, the convenience element of hand sanitizer products, such as color, aroma, and texture, must be prioritized, as well as the absence of adverse effects on the skin. According to Fauziati (2012), liquid smoke cannot be used as a hand antiseptic because of its color and odor.

CONCLUSION

Pine fruit liquid smoke (*Pinus merkusii*) is effective as an antibacterial against the growth of *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 at a concentration of 100%, generating an inhibition zone of 5.3 mm and 5.8 mm, respectively. The in vitro test results are corroborated by the in vivo test results, which indicate that the liquid smoke of pine fruit is 100% effective at preventing the growth of bacteria by 75.1% and fungi by 87.3%.

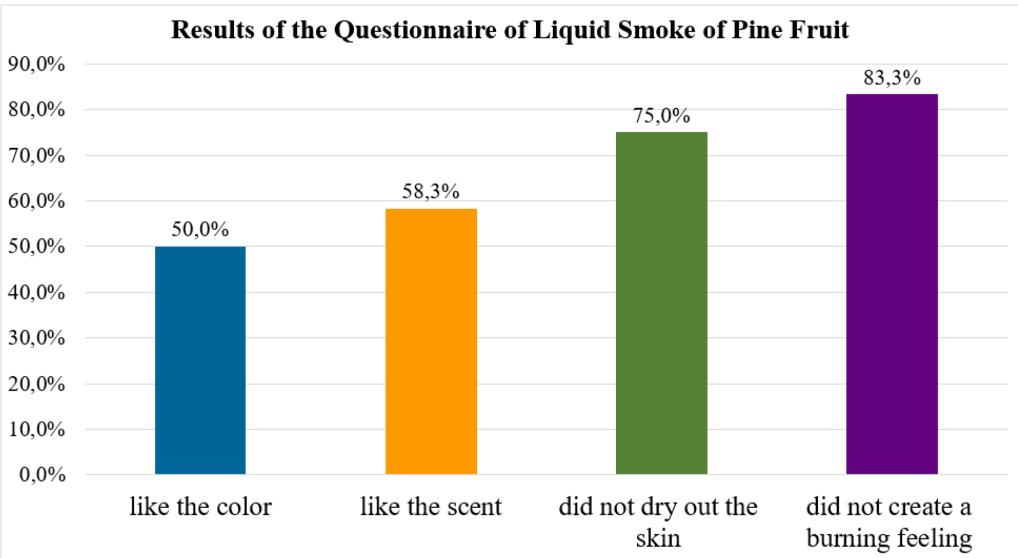

Figure 4. Results of the Questionnaire of Liquid Smoke of Pine Fruit as an Antiseptic

REFERENCES

Agnol, R. D., Ferraz, A. Z., Bernardi, A. P., & Albring, D. (2003). *Antimicrobial Action of Some Hypericum Species.* Brazil: TANACSA. https://doi.org/10.1078/094471103322331476

Aisyah, I. (2019). *Manfaat Arang dan Asap Cair dari Limbah Biomasa.* Yogyakarta: Deepublish. https://opac.perpusnas.go.id/DetailOpac.aspx?id=1227424#

Aisyah, I., Giyanto, Sinaga, M. S., Nawangsih, A. A., & Pari, G. (2018). Uji In vitro Asap Cair terhadap Ralstonia syzygii subsp. celebesensis Penyebab Penyakit Darah pada Pisang. *J Fitopatol Indones, 14*(4), 145-151. https://doi.org/10.14692/jfi.14.4.145

Asngad, A., Aprilia, B. R., & Nopitasari. (2018). Kualitas Gel Pembersih Tangan (Handsanitizer) dari Ekstrak Batang Pisang dengan Penambahan Alkohol, Triklosan dan Gliserin yang Berbeda Dosisnya. *Bioeksperimen, 4*(2), 61-70. https://doi.org/10.23917/bioeksperimen.v4i2.6888

Aznury, M., Sofiah, & Sari, R. P. (2020). Produk Gel Hand Sanitizer Berbahan Dasar Ekstrak Cair Daun Sirih Hijau (Piper betle Linn.) sebagai Antiseptik. *Jurnal Kinetika, 11*(1), 27-35. https://jurnal.polsri.ac.id/index.php/kimia/article/view/3107

Bertrand, B. L. (2019). Lag Phase is a Dynamic, Organized, Adaptive, and Evolvable Period That Prepares Bacteria For Cell Division. *Journal of Bacteriology, 201*(7), 1-21. https://doi.org/10.1128/jb.00697-18

Bowman, S. M., Piwowar, A., Dabbous, A. M., Vierula, J., & Free, S. J. (2006). Mutational Analysis of the GPI Anchor Pathway Demonstrates that GPI Anchored Proteins are Required for Cell Wall Biogenesis and Normal Hyphal Growth in Neurospora crassa. *Eukaryotic Cell, 5*, 187-200. https://doi.org/10.1128/ec.5.3.587-600.2006

Dediwanto, Y., Oramahi, H. A., & Nurhaida. (2020). Potensi Asap Cair Dari Kayu Bengkirai dalam Menghambat Pertumbuhan Jamur Schyzophylllum commune Secara In vitro. *J Galung Tropika, 9*(1), 1-9. https://doi.org/10.31850/jgt.v9i1.518

Fauziati, (2012). Pemanfaatan Asap Cair dari Cangkang Kelapa Sawit sebagai Bahan Antiseptik Pembersih Tangan Asap Cair dari Cangkang Sawit sebagai Bahan Baku Industri. *Jurnal Riset Teknologi Industri, 6*(12), 11-19. http://dx.doi.org/10.26578/jrti.v6i12.1513

Hasanah, U. (2017). Mengenal Aspergillosis, Infeksi Jamur Genus Aspergillus. *Jurnal Keluarga Sehat Sejahtera, 15*(2), 76-86. https://doi.org/10.24114/jkss.v15i2.8777

Hutasoit, D. P. (2020). Pengaruh Sanitasi Makanan dan Kontaminasi Escherichia coli terhadap Penyakit Diare. *Jurnal Kesehatan Sandi Husada, 9*(2), 779-786. https://doi.org/10.35816/jiskh.v12i2.399

Kalista, K. F., Chen, L. K., Wahyuningsih, R., & Rumende, C. M. (2017). Karakteristik Klinis dan Prevalensi Pasien Kandidiasis Invasif di Rumah Sakit Cipto Mangunkusumo. *JPDI, 4*(2), 56-61. http://dx.doi.org/10.7454/jpdi.v4i2.104

Kusuma, Y., Pinatih, K. P., & Hendrayana, M. A. (2019). Efek Sinergis Kombinasi Chlorhexidine dan Alkohol Terhadap Daya Hambat Pertumbuhan Staphylococcus aureus. *E-Jurnal Medika, 8*(3). https://ojs.unud.ac.id/index.php/eum/article/view/48937

Lan, H., Wu, L., Sun, R., Yang, K., Liu, Y., Wu, J., et al. (2018). Investigation of Aspergillus flavus in Animal Virulence. *Toxicon, 14*(5), 40-47. https://doi.org/10.1016/j.toxicon.2018.02.043

Mahjani, & Putri, D. H. (2020). Growth Curve of Endophyte Bacteria Andalas (Morus macroura Miq.) B.J.T. A-6 Isolate. *Serambi Biologi, 5*(1), 29-32. http://dx.doi.org/10.24036/5692RF00

Makhfirah, N., Fatimatuzzahra, C., Mardina, V., & Hakim, R. F. (2020). Pemanfaatan Bahan Alami Sebagai Upaya Penghambat Candida albicans pada Rongga Mulut. *Jurnal Jeumpa, 7*(2), 400-413. https://doi.org/10.33059/jj.v7i2.3005

Mardalena. (2016). Fase Pertumbuhan Isolat Bakteri Tahan Asam Laktat (BAL) Tempoyak Asal Jambi yang Disimpan pada Suhu Kamar. *JSPI, 1*(1), 58-66. https://doi.org/10.31186/jspi.id.11.1.58-66

Muharini, F. & Farida, S. (2017). Uji Aktivitias Antibakteri Ekstrak Etanol Tanaman Obat Suku Musi di Kabupaten Musi Banyuasin Sumatera Selatan. *Jurnal Kefarmasian*, *7*(2), 127-135. https://doi.org/10.22435/jki.v7i2.3493

Pragita, A. S., Shafa , D. P., Nursifah, D., Rumidatul, A., Fadhila, F., & Maryana, Y. (2020). Uji Aktivitas Antimikroba Ekstrak Kulit dan Kayu Sakit Ranting Sengon Terhadap Bakteri dan Jamur. *J Analis Kesehatan, 9*(2), 41-48. https://www.ejurnal.poltekkes-tjk.ac.id/index.php/JANALISKES/article/view/2459

Pudja, I. R., Kencana, P. D., & Jakung, M. Y. (2020). Pengaruh Konsentrasi Asap Cair Bambu Tabah (Gigantochloa nigrociliata Buse-Kurz) dan Suhu Pemasakan terhadap Mutu Se'i Bandeng. *J BETA, 8*(1), 93-102. https://doi.org/10.24843/JBETA.2022.v10.i01.p16

Pulungan, A. S. (2017). Aktivitas Antijamur Ekstrak Etanol Daun Kunyit (Curcuma longa LINN) terhadap Jamur Candida albicans. *Jurnal Biollink, 3*(2), 120-124. https://doi.org/10.31289/biolink.v3i2.843

Purwaningsih, D. & Wulandari, D. (2021). Uji Aktivitas Antibakteri Hasil Fermentasi Bakteri Endofit Umbi Talas (Colocasia esculenta L) terhadap Bakteri Pseudomonas aeruginosa. *J Sains Kes, 3*(5), 750-9. https://doi.org/10.25026/jsk.v3i5.622

Risna, Y. K., Harimurti, S., Wihandayo, & Widodo. (2022). Kurva Pertumbuhan Bakteri Asam Laktat dari Saluran Pencernaan Itik Lokal Asal Aceh. *JPI, 24*(1), 1-7. https://doi.org/10.25077/jpi.24.1.1-7.2022

Senjaya, & Surakusumah. (2007). Potensi Ekstrak Daun Pinus (*Pinus merkusii*) sebagai Bioherbisida Penghambat Perkecambahan Echinochloa colonum L dan Amaranthusviridis. *Jurnal Parrenial, 4*(1), 1-5. https://doi.org/10.24259/perennial.v4i1.175

Setyati, W. A., Martani, E., Subagiyo , T., & Zainuddin, M. (2015). Kinetika Pertumbuhan dan Aktivitas Protease Isolat 36k dari Sedimen Ekosistem Mangrove, Karimunjawa, Jepara. *J Ilmu Kelautan, 20*(3), 163-169. https://doi.org/10.14710/ik.ijms.20.3.163-169

Sharah, A., Karnila, R., & Desmelati. (2015). Pembuatan Kurva Pertumbuhan Bakteri Asam Laktat yang Di Isolasi Dari Ikan Peda Kembung (Rastrelliger sp.). *JOM, 2*(2), 1-8. https://jom.unri.ac.id/index.php/JOMFAPERIKA/article/view/6153

WHO. (2016). *The Burden Of Health Care-Associated Infection Worldwide*. https://www.who.int/gpsc/country_work/gpsc_ccisc_fact_sheet_en.pdf (Diakses 20 Januari 2021).

WHO. (2017). *Monitoring Health For SDSGs, Suitanable Development Goals*. https://apps.who.int/iris/bitstream/handle/10665/255336/9789241565486-eng.pdf?sequence=1&isAllowed=y (Diakses 20 Januari 2021)