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Effectiveness of Tea Twig Liquid Smoke (*Camellia sinensis*) as an Antiseptic Against Microbes in Vivo and In Vitro

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Abstract. Transmission of microorganisms that cause disease in humans occurs directly or indirectly. Prevention efforts can use antiseptics. The content of liquid smoke compounds in tea twigs has the potential to be an antiseptic. The purpose of this study was to determine the effectiveness of liquid smoke from tea twigs (*Camellia sinensis*) on microbial growth in vitro using the inhibition test method and in vivo swab test on the palms. In the in vitro test, the most effective results were grade 2 liquid smoke with a concentration of 75% with an inhibition zone diameter of 18.1 mm (E. coli ATCC 25922), 15.6 mm (S. aureus ATCC 25923), 32.3 mm (A. flavus ATCC 9643), 4.3 mm (C. albicans ATCC 10231). The results of the in vivo test showed effectiveness in reducing the number of bacterial colonies by an average of 88.33%, and fungal colonies by an average of 91.52%. From the entire questionnaire data, the parameters of aroma, color, dryness are in the criteria of liking and do not cause side effects on the skin. The results of the study showed that liquid smoke from tea twigs has the potential as an antiseptic.

Keywords: Liquid Smoke; Tea Twigs; Antiseptic

INTRODUCTION

Transmission of disease-causing microorganisms to humans can occur directly or indirectly. Microorganisms that can cause infection are bacteria and fungi. *Escherichia coli* is known as one of the bacteria that cause intestinal infections, especially diarrhea (Amelia & Burhanuddin, 2018). According to the Indonesian Ministry of Health, Extraordinary Events (KLB) to be precise in 2017 there have been records of diarrheal diseases occurring 21 times which have spread to 12 provinces and 17 districts / cities with 1725 patients and 34 deaths (Adhiningsih et al., 2019). Another bacterium that can cause infection is *Staphylococcus aureus* which is a trigger for nosocomial infections. Indonesia itself has a nosocomial infection prevalence record of 7.1% (Amelia & Burhanuddin, 2018). *Staphylococcus aureus* can also be the cause of cases of Acute Respiratory Infection (ARI). In a study by Rudan et al, stated that the latest cases of ARI in the world amounted to 156 million per year. Among these numbers, 151 million cases (96.7%) were found in developing countries (Aristatia, 2021). If ARI treatment is not carried out properly, it can lead to complications that are classified as serious, such as the emergence of infections in the lungs, infections in the membranes of the brain, decreased awareness, respiratory failure, and even death, especially in infants and toddlers who do not have optimal immune strength (Amila et al., 2021).

Aspergillosis is the term refers to diseases of the respiratory system caused by the presence of fungal infections of the genus Aspergillus. Specialist causes are cosmopolitan including *Aspergillus flavus* (A. flavus) (Hasanah, 2017). The Medical Records Installation of Dr. Sarjito Hospital said there were 902 cases of otomycosis found in the city of Yogyakarta between 2014 and December 2018. Otomycosis disease is caused by the growth of foreign substances from the genus Aspergillus. One of the fungal infections that has been widely spread in Indonesia is Candidiasis. The main cause of the fungal infection is the agent Candida sp (Widaty, 2016). A number of epidemiological studies conducted in Hong Kong explained that the number of candidiasis incidence rates found in the Asian region was found to originate from C. albicans which is a species most often known to be the cause with an average of 56% of candidiasis cases. C. albicans as the dominant

species has had incidence rates ranging from 37% in Latin America to 70% in Norway. This has been the result of an increase in the incidence of invasive candidiasis along with an increase in the population of susceptible individuals, and the hindrance of treatment by antifungal resistance (D'Enfert et al., 2021).

Efforts to prevent disease transmission can be done with the habit of washing hands using soap (Huliatunisa et al., 2020). The results of global research show that the use of soap for hand washing habits can not only minimize the level of disease transmission but can also be useful as a prevention of the incidence of diarrheal diseases by 50% and ARI by 45%, according to (Yannuarista et al., 2020) alcohol, glycerol, tricholosan and other chemicals contained in hand sanitizers can cause side effects in the form of dryness and irritation located on the skin which generally occurs in children. Therefore, an alternative to natural ingredients is needed as a substitute for hand sanitizer ingredients, one of which is tea twigs which has the potential as an anti-microbial. Green tea stores the largest content in the form of flavonoid compounds which are referred to as a class of polyphenolic compounds (Tea, 2018). Referring to the results of phytochemical tests conducted using ethanol solvents, it is stated that tea twigs contain flavonoid compounds and catechins (Hung et al., 2021). It can be known that flavonoids are active compounds that can be utilized as antibacterial, anti-inflammatory, and even antifungal. The process of flavonoids in inhibiting fungal growth can trigger disruption of fungal cell membrane permeability (Asngad et al., 2018).

The content of compounds in tea twigs can be obtained by pyrolysis method, the result of the pyrolysis process used is liquid smoke. The term liquid smoke can be called liquid smoke which is intended as a result of condensation or condensation from the evaporation process from combustion carried out either directly or indirectly derived from various materials that contain lignin, cellulose, hemicellulose and other carbon compounds that can bring various compounds such as phenols, carbonyls and so on. From several studies, it is said that liquid smoke is used as anti-bacterial, anti- fungal, anti-insect, and anti-termite (Oramahi et al., 2018).

Liquid smoke is unique in that it can have antibacterial properties. The existence of properties that function as antibacterial has a relationship with the content of phenol and acetic acid contained in liquid smoke. The ability of phenol compounds is believed to cause damage to the cytoplasmic membrane resulting in leakage of the membrane which can interfere with bacterial growth and can even result in death. In addition, acetic acid can also cause destabilization of various functions and structures in cell components (Fitri et al., 2024). The results of research by (Indah Lestari, 2017), show that the extract produced from tea leaves can be used as an inhibitor of Aspergilus flavus growth. Based on the description above, it shows that tea twig liquid smoke has the potential to inhibit the growth of bacteria and fungi, so further research needs to be done so that the benefits of tea twig liquid smoke inhibit the growth of bacteria and fungi can be known in more detail. The purpose of this study was conducted as a means to see whether there is an effectiveness of tea twig liquid smoke on microbial growth in vitro which is used as an antiseptic with the help of an inhibition test and in vivo which is carried out by the swab test method on the palm of the hand.

MATERIALS AND METHODS

The method used in this study is to use an experimental descriptive method, and carry out testing at the Microbiology Laboratory of the Rajawali Institute of Health Bandung from January to February 2022 by means of in vitro and in vivo.

In the implementation of this study, the samples used in the in vitro test were *Escherichia coli* ATCC 25922, Candida albicans ATCC 10231, *Staphylococcus aureus* ATCC 25923, and *Aspergillus flavus* ATCC 9643 tested with tea twig liquid smoke (*Camellia sinensis*) at concentrations of 35%, 50%, and 75%. The number of samples used in in vivo testing amounted to 24 respondents consisting of men and women, each of which amounted to 12 respondents. Related to the Swab test through the palm of the respondent is carried out before the start and after the action is given to the liquid smoke of the optimum concentration of tea twigs from the in vitro test. In determining the number of respondents determined using the federer formula.

Research tools and material

The research used various tools, namely erlenmeyer, autoclave, oven, petri dish, test tube, stirring rod, cotton swab, paper disc, micropipette, vernier, colony counter, and pH meter. The materials used were grade 1 and grade 2 tea twig liquid smoke, 70% alcohol, sterile distilled water, nutrient agar medium, potato dextrose agar medium, 1% orange scent perfume, gram staining reagent, lactophnenol cotton blue staining reagent, Candida albicans ATCC 10231, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Aspergillus flavus* ATCC 9643 cultures.

Data processing and analysis

Effectiveness results from a decrease in the number of microbes. Questionnaire data in the form of secondary data obtained from respondents with several questionnaire parameters including preference for color, aroma, dryness effect, itching effect and burning. The questionnaire assessment used has several points, namely 0 for dislike, 1 is normal, 2 likes, and 3 is very like. There are criteria for respondents intended in this study, namely focusing on respondents who have not washed their hands using water or soap, do not have allergies to hand sanitizer or alcohol, do not have wounds on their hands.hand parts, and have the willingness to fill out a questionnaire sheet. The results obtained are in the form of calculating the number of microbial colonies that grow on NA and PDA medium. Measurements were made by comparing the growth of the number of microbial colonies from swab samples before and after being given 75% tea twig liquid smoke natural ingredient hand sanitizer. Then compared with the control results, namely using an antiseptic containing 70% alcohol. Then the data obtained is processed using the help of a computer program, namely Microsoft excel to determine the correlation results of the use of 75% tea twig liquid smoke hand sanitizer.

RESULTS AND DISCUSSION

The observation of the characteristics of the tea twig liquid smoke solution is macroscopically seen in terms of color, which is yellowish brown with a pungent smoke aroma, and the consistency of the solution is liquid and has a pH of 2.6.

Table 1. Macroscopic Characteristics of Tea Twig (*Camellia sinensis*) Liquid Smoke

Physical Properties	Liquid smoke from tea twigs 75% concentration	Picture
Color	Yellowish brown	
Aroma	Stinging	
Consistency	Liquid	
Claritty	Somewhat clear	
pH	2,6	

Microbial Identification

Table 2. Microbial Identification

Type of Microbe	Clony Morphology	Cell Morphology
Escherichia coli ATCC 25922	Colonies are small round in shape, convex in elevation, smooth in texture and milky white/opaque in color	Short, red rods are Gram negative
Staphylococcus aureus ATCC 25923	The colonies are round and resemble grapes in shape, convex in elevation, and white in color	Round and clustered like grapes and purple are Gram positive
Aspergillus flavus ATCC 9643	Colonies are green to yellowish green with a granular and compact colony shape	Round to oval vesicles, round conidia, conidiophores long, cylindrical
Candida albicans ATCC 10231	Colonies are small round in shape white mucoid texture with smooth and even edges	Oval in shape, purple in color, Gram positive

Microbial identification was carried out on *Escherichia coli* ATCC 25922, Candida albicans ATCC 10231, *Staphylococcus aureus* ATCC 25923, and *Aspergillus flavus* ATCC 9643 carried out macroscopically and microscopically the results can be seen in Table 2.

In Vitro Test Results

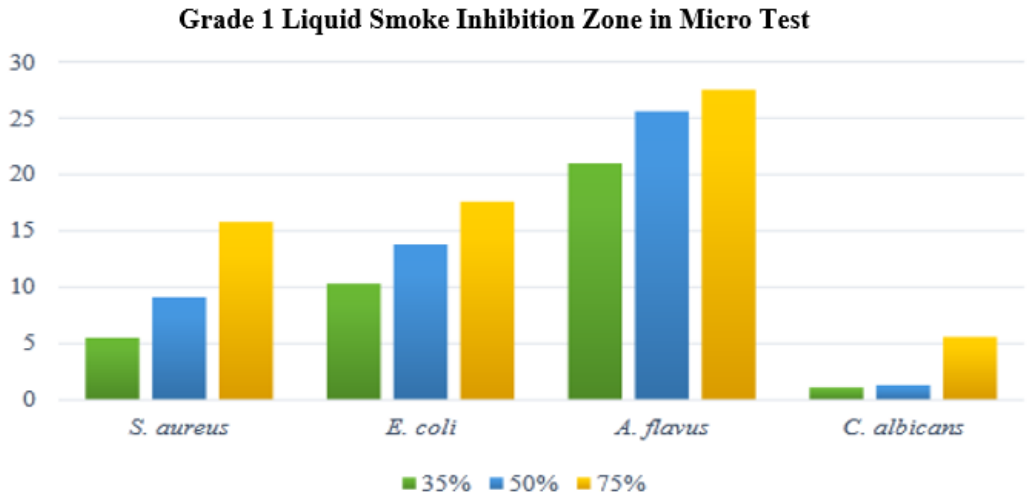

Figure 1. Zone of Inhibition of Liquid Smoke Tea Twigs (*Camellia sinensis*) Grade 1 on Test Microbes

Based on Figure 1, the results of the in vitro test showed that grade 1 liquid smoke of tea twigs (*Camellia sinensis*) can inhibit the growth of E. coli ATCC 25922 with a concentration of 35% with a size of 10.3 mm, 50% concentration with a size of 13.8 mm, and 75% concentration with a size of 17.6 mm. In vitro test results on S. aureus ATCC 25923, 35% concentration with a size of 5.5 mm, 50% concentration with a size of 9.1 mm, and 75% concentration with a size of 15.8 mm using the disc diffusion method. Then the results of inhibition on the growth of A. flavus ATCC 9643 35% concentration with a size of 21 mm, 50% concentration with a size of 25.6 mm, and 75% concentration with a size of 27.5 mm. In vitro test results on C. albicans ATCC 10231 35% concentration with a size of 1.1 mm, 50% concentration with a size of 1.3 mm, and 75% concentration with a size of 5.6 mm using the well diffusion method.

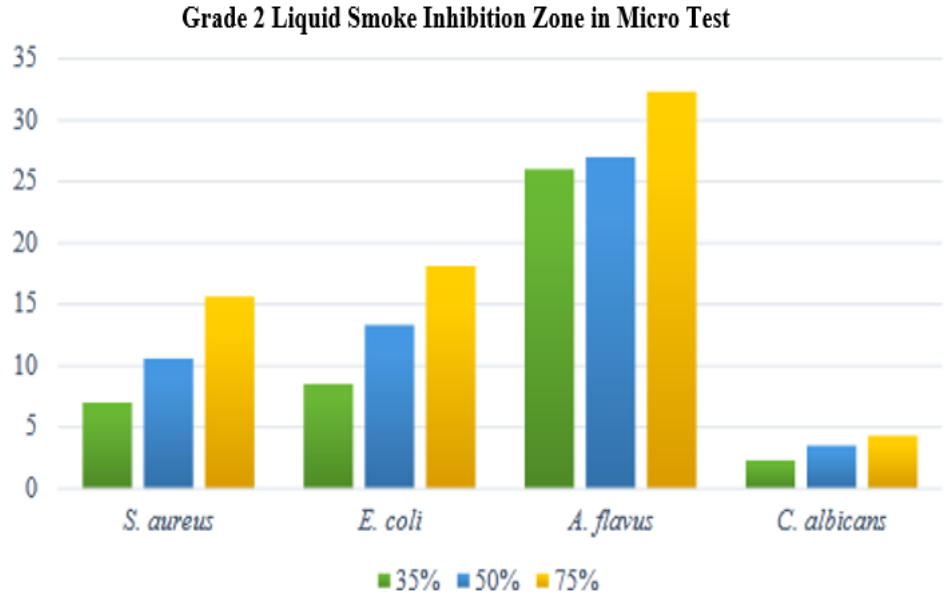

Figure 2. Zone of Inhibition of Liquid Smoke Tea Twigs (*Camellia sinensis*) Grade 2 on Test Microbes

Figure 2 shows the results of the in vitro test of liquid smoke of tea twigs (*Camellia sinensis*) grade 2 can inhibit the growth of *E. coli* ATCC 25922 35% concentration with a size of 8.5 mm, 50% concentration with a size of 13.3 mm, and 75% concentration with a size of 18.1 mm. In vitro test results on *S. aureus* ATCC

25923 35% concentration with a size of 7 mm, 50% concentration with a size of 10.6 mm, and 75% concentration with a size of 15.6 mm using the disc diffusion method. Then the results of inhibition on the growth of *A.flavus* ATCC 9643 35% concentrationwith a size of 26 mm, 50% concentration with a size of 27 mm, and 75% concentration with a size of 32.3 mm. In vitro test results on *C. albicans* ATCC 10231 35% concentration with a size of 2.3 mm, 50% concentration with a size of 3.5 mm, and 75% concentration with a size of 4.3 mm using the well diffusion method.

In Vivo Test Results

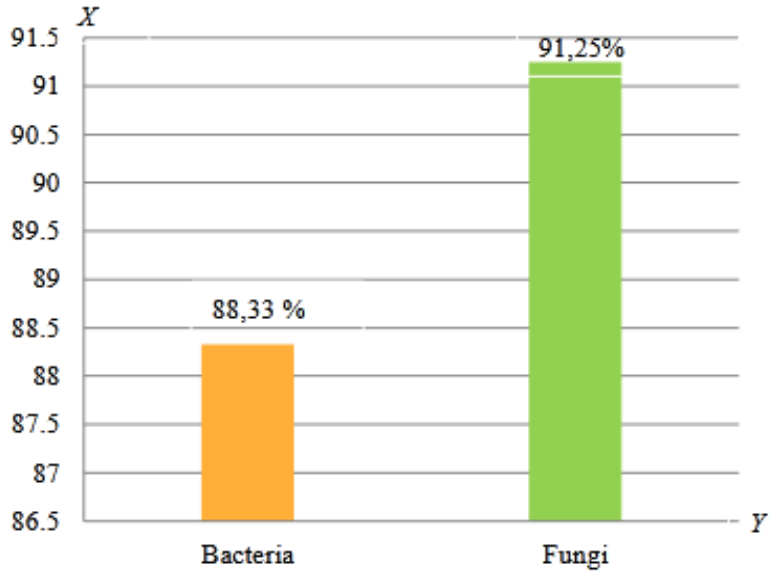

Figure 3. Liquid Smoke Effectiveness Tea Twigs

Based on Figure 3, it shows the results of the effectiveness of 75% tea twig liquid smoke in inhibiting microbial growth with the in vivo method, and the results obtained have an average percentage decrease in the number of bacteria by 88.33% and fungi by 91, 25, this indicates that 75% concentration of tea twig liquid smoke is able to inhibit microbial growth.

Questionnaire Results

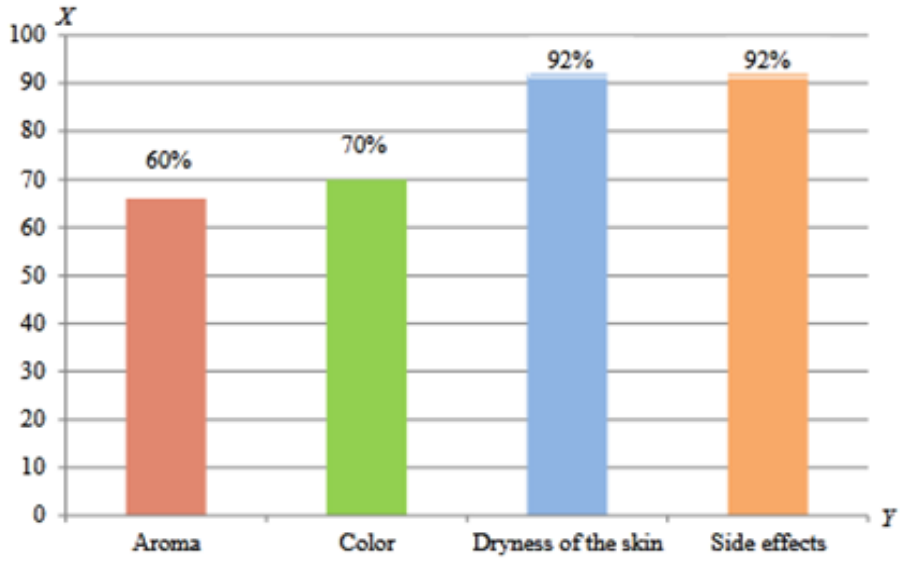

Figure 4. Percentage of Smoke Characteristics of Liquid Tea Twigs Overall Preferred Respondents

Questionnaire data collection is carried out with the aim of measuring the level of general public preference as a co-respondent to tea twig liquid smoke products that are used as ingredients for making antiseptics. Based on Figure 4, the results show that 66% do not mind the aroma of tea twig liquid smoke which has been added with 1% citrus aroma perfume, the addition of this perfume aims to disguise the aroma like burnt results but 34% of respondents do not like the aroma of antiseptic made from 75% tea twig liquid smoke, this is because it can still smell smoke after a while after use and mixed with citrus fragrance which begins to disappear.

Based on the results of measuring the inhibition zone in the liquid smoke of the twig (*Camellia sinensis*) with concentrations of 35%, 50%, and 75% can inhibit the growth of A. flavus ATCC 9643 and C. albicans ATCC 10231. The inhibition zone contained in each concentration is due to the presence of phenol compounds that can cause antifungal effects in tea twig liquid smoke because if the higher levels of phenol in liquid smoke cause its antifungal activity to increase as well. In inhibiting fungal growth, phenol denatures cell proteins and damages cell membranes, so do not be surprised if it causes death to pathogens (Mayasari, 2020). Flavonoid compounds have a role as antifungals aimed at forming a complex compound and forming extracellular proteins and soluble proteins that cause disruption of fungal cell membrane permeability. Not only that, the ability of flavonoids can also inhibit the work of enzymes that have a role in the synthesis of fungal cell walls such as mannan synthase, chitin synthase, and glucan synthase. If there are inhibitors when these enzymes work, it will certainly interfere with the process of forming fungal cell walls and inhibit the process of pseudohyphae formation (Mayasari, 2020). As for the results of the measurement of the inhibition zone against liquid smoke of tea twigs (*Camellia sinensis*) with concentrations of 35%, 50%, and 75% can inhibit the growth of E. coli ATCC 25922 and S. aureus ATCC 25923. The occurrence of the inhibition zone is caused by the presence of phenol compounds.

The results of the analysis of the characteristics of tea twig liquid smoke obtained that grade 2 tea twig liquid smoke has a slightly cloudy clear yellow color and has better antifungal properties than grade 1 tea twig liquid smoke because it contains very high acids and phenols and has a very low pH level value compared to grade 1 tea twig liquid smoke. Grade 1 tea twig liquid smoke has a clear yellow color due to the separation of harmful compounds and high tar in tea twig liquid smoke (*Camellia sinensis*). It is explained that the pH of good liquid smoke is in the range of 1.5-3.7 because in low pH conditions fungi with spores cannot live and multiply so that they can potentially inhibit the growth rate of fungi (Pujilestari, 2015).

The content of active compounds contained in liquid smoke consists of phenol compounds and organic acids with anti-microbial abilities (Erlytasari et al., 2019). In addition to phenol, liquid smoke also contains other compounds in the form of acetic acid. The acidic antimicrobial effect of liquid smoke may directly shape a cytoplasm to be acidic, damaging the surface tension of the membrane and the loss of active transport of food through the membrane, causing destabilization of many types of functions and structures of cell components. Acidic compounds can also interfere with bacterial cell metabolism by lowering the pH of bacterial cells.

Phenol compounds contained in liquid smoke have the ability to damage the protein structure of bacteria by means of hydrogen bonds, so that bacterial metabolic activity will stop (Sampepana & Fauziati, 2021). According to (Imas et al., 2018), the mechanism of alcohol compounds in inhibiting microbial growth, namely that in bacteria alcohol compounds react with lipid proteins, then will denature the proteins that make up the cell membrane and bacterial cell wall, while in fungi it will denature the fungal conidia membrane and dissolve the lipids that make up the hyphal cytoplasmic membrane and hyphal cell wall. Acidic compounds contained in liquid smoke can break through the cell walls of microorganisms, causing the cells of microorganisms to lysis.

Tea twig liquid smoke with a concentration of 75% has the ability to inhibit microbial growth. Based on (Kondo et al., 2017), it states that the higher the concentration level in the liquid smoke solution, the stronger the ability to inhibit bacterial growth. Supported by (Mayasari, 2020), liquid smoke without purification or without dilution has strong inhibition against three types of bacteria, namely *Escherichia coli*, *Salmonella* sp., and *Staphylococcus aureus*. In a study conducted by (Aznury et al., 2021), that people in choosing hand sanitizers prefer hand sanitizers with a very non-pungent aroma, this is because people do not really like the smell of hand sanitizers that are very pungent.

Most respondents were interested in the color of the 75% concentration of tea twig liquid smoke, because it was yellowish brown, slightly transparent. This is supported by the research of (Aznury et al., 2021), that the clear transparent color of the hand sanitizer gel is the most favorite color choice, this is because it has attractiveness and is brighter in terms of color, while the dark color is less attractive to the public. Color selection for a product has an important role in order to be of interest to many people. It is known that most people feel like the dryness caused after the use of 75% tea twig liquid smoke and only a small percentage feel dislike the dryness of 75% tea twig liquid smoke. People feel more comfortable when using products that provide a cool feeling and are not too sticky when not in use, and prefer hand sanitizers with gel texture compared to liquid texture (Aznury et al., 2021).

Figure 4 shows that most people 92% like the sensation effect caused, because they do not get side effects from using 75% tea twig liquid smoke hand sanitizer. Based on the color and aroma arising from the liquid smoke of tea twigs, it is still not possible as an antiseptic that people like. The use of hand sanitizer products is sticky, feels hot, causes irritation, it will interfere with the health of its users. So that the comfort factor of using hand sanitizer products must be prioritized such as in terms of color, aroma, texture, and the absence of side effects caused on the skin.

CONCLUSION

Based on the results of research that has been done , it can be concluded that grade 2 tea twig liquid smoke with a concentration of 75% has a better ability to inhibit the growth of bacteria and fungi.

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