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Original Article

The Antifungal Effect of WHO Hand Sanitizer to *Candida Albicans* in Improving Personal Hygiene

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ABSTRACT

Hand hygiene is one of the important factors that determine a person's health status. Hands are easily contaminated by microbes from the environment such as bacteria, viruses, and fungi through direct contact. Hand sanitizer is a type of media that can be used to clean hands from disease-causing microbes other than soap. Hand sanitizers are widely used by the community because they are considered more practical to use. This study aims to determine the anti-fungal activity of the hand sanitizer recommended by the World Health Organization (WHO) against Candida albicans. Anti-fungal activity was tested using the well diffusion method. The results showed that the higher the concentration of the hand sanitizer, the bigger the inhibition zone formed. The statistical test results obtained a significance value of p <0.05 at the variation of the concentration of hand sanitizer 50%, 75%, 100%, and treatment control. These results indicate that there is a significant difference between the hand sanitizer treatment and control of the inhibition zone formation in C. albicans. The WHO recommended hand sanitizer has medium inhibitory power against C. albicans bacteria.

Keywords: anti-fungal, inhibitory zone, hand disinfectants, hand hygiene, hand sanitizer

BACKGROUND

Hands are one part of the body that can be a medium for microbial transmission and are considered as the main cause of infection in hospitals (1,2). Hands are easily contaminated with microbes through direct contact with air or objects (3). Microbes like bacteria, viruses, and fungi can stick to someone's hands when doing activities or activities (1). Microbes, especially fungi, have the potential to become pathogens that caused infection and severe disease for humans (4). Candidiasis is one of the most common cases of fungal infection in humans (5). Candidiasis is an opportunistic infection caused by an overgrowth of the fungal genus *Candida*, 70% of *Candida* infections are caused by *Candida albicans*. In the human body, *Candida* fungi can live as parasites or saprophytes in the mouth, respiratory tract, digestive tract, or vagina (5). Candidiasis vaginalis is a vaginal infection caused by *Candida* sp. especially *C. albicans* (5). Infection occurs due to changes in vaginal conditions due to the use of broad-spectrum antibiotics, use of contraceptives, high estrogen levels, pregnancy, uncontrolled diabetes, wearing tight clothing, and high sexual frequency (5). Candidiasis vaginalis affects 75% of women at some point in their life and 10-20% of women are asymptomatic carriers for the Candida species (5).

Maintaining hand hygiene is an effective basic step to avoid disease and limit germ-to-human transmission (1,3). The easiest and simplest but effective way to maintain hand hygiene is to wash your hands using antiseptic soap and running water (1). Washing hands with antiseptic soap can remove dirt and reduce the number of germs on the hands, besides washing hands prevents a person from various diseases transmitted through hands such as diarrhea, worms, and cholera (1,6). In certain conditions, the unavailability of water and soap can be an obstacle to cleaning hands, therefore the use of hand sanitizers to clean hands is increasing and more attractive to the public because it is

considered more practical to use and easy to carry (1,6). The occurrence of the Covid-19 pandemic has also increased the use of hand sanitizers (HS) in the community (3).

In general, hand sanitizer is divided into two groups: hand sanitizer alcohol-based hand sanitizer alcohol-free (3). Hand sanitizers alcohol-based generally contain active substances in the form of alcohol (60-95%) and other antimicrobial substances such as triclosan (0.05-25%) which can denature and coagulate microbial proteins and inactivate viruses (3,6,7). Hand sanitizers Alcohol-based can be found in various forms, namely gel, liquid (liquid), and foam (foam), each of which has different characteristics (3).

In March 2020, the Food and Drug Supervisory Agency (BPOM) issued a Circular Letter Number KP.11.01.2.83.03.20.14 which explains how to formulate hand sanitizer independent according to the guidelines of the World Health Organization (WHO). This study aims to determine the anti-fungal activity of hand sanitizer, the WHO standard against *C. albicans.*

MATERIALS AND METHOD

This research was conducted at the Laboratory of Microbiology, Molecular Biology, and Immunology (MBI), Banjarnegara Health Research and Development Center in July 2020. The study was a laboratory experimental study with a completely randomized design or CRD. The materials used were test fungi *Candida albicans*, hand sanitizer (96% ethanol, 98% glycerol, 3% hydrogen peroxide (H_2O_2), and sterile distilled water), and NA medium.

Hand sanitizers are made based on the Circular Letter of the Food and Drug Supervisory Agency (BPOM) number KP.11.01.2.83.03.20.14 concerning the Making of Hand Sanitizers in Efforts to Prevent Coronavirus. A total of 833 mL of 96% ethanol was put into a 1000 mL measuring cup, then 41.7 mL of added H2O2 3% and 14.5 mL of 98% glycerol were. Then add distilled water up to 1000 mL and stir until homogeneous. The Hand sanitizer is then transferred to a clean bottle and stored for a minimum of 72 hours to ensure there is no contamination of organisms from the bottle. The Hand sanitizer is then diluted with sterile distilled water to produce a series of concentrations, namely 25%, 50%, 75%, and 100%. The negative control used sterile aquadest while the positive control used hand sanitizer Gel MjSM-Central Java which was diluted with sterile distilled water (5% concentration).The fungal culture medium used was the Potato Dextrose Agar (PDA) medium, which was made according to the determined composition. The preparation of the mushroom suspension used in the test was carried out by dissolving *C. albicans* in NaCl solution, which was then tested for its turbidity with Mc. Farland 0.5% (1% sulfuric acid 9.95 mL and 1% Barium chloride 0.05 mL). After obtaining the appropriate turbidity, the bacterial suspension was inoculated in the agar medium by the method pour plate (20 mL agar volume and 500 mL bacterial suspension volume). The antibacterial test method used is the method well diffusion. The well on the agar medium in a petri dish is made by perforating the agar with a gel cutter (6 mm in diameter). In the treated petri dishes 5 wells were made for control - and hand sanitizer concentrations of 25%, 50%, 75%, 100%, while the control + was made on different petri dishes. The number of replications used in this study were five replications for each concentration of treatment and control. Incubation was carried out in an incubator with a temperature of 37^0C.

Observation and measurement of the diameter of the inhibition zone or clear zone formed around the well is carried out after 24 hours. Furthermore, the data obtained were analysed using the statistical test One-way anova. If the results show a significant or significant difference if the p-value is <0.05 then the test is continued with the post hoc test.

RESULT

The inhibition of bacterial growth which shows the antibacterial activity of the *hand sanitizer* can be seen from the formation of an inhibition zone or a clear zone around the well (Figures 1). Based on Figures 1, it is known that all treatments with various concentrations of *hand sanitizer* can inhibit the growth of *C. albicans.*

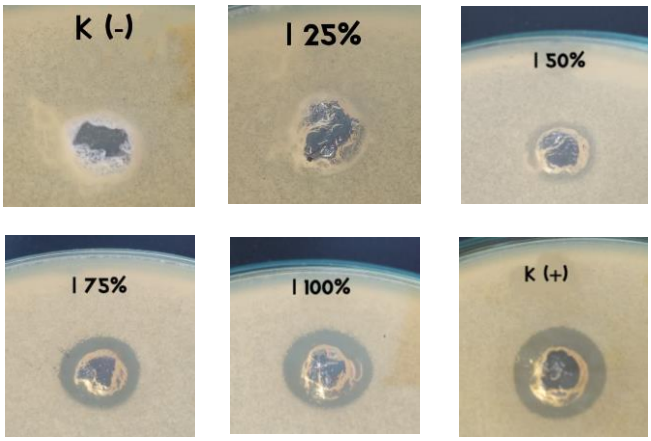

Figure 1. Inhibition zone formed by HS treatment

The diameter of the inhibition zone produced at each concentration of hand sanitizer was different (Table 1). Based on Table 1, it is known that the higher the concentration hand sanitizer, the wider the diameter of the inhibition zone formed and statistically the results of each treatment show a significant// significant difference except the concentration of the hand sanitizer 25% and control -.

Table 1. Killing zone of Hand Sanitizer (HS) towards *Candida albicans*

Treatment	Mean of kill zone (mm) ± standard deviation
Control +	12,80 ± 0,45[a]
Control -	0,00 ± 0,00[b]
HS 25%	0,00 ± 0,00[b]
HS 50%	10,00 ± 0,00[c]
HS 75%	12,00 ± 0,71[d]
HS 100%	13,60 ± 0,55[e]

Note = numbers followed by different letters in the same column indicate significant / significant differences (α = 0,05)

Table 1 shows that the mean diameter of the widest inhibition zone in *C. albicans was* formed on exposure to hand sanitizer with a concentration of 100%. Based on the analysis One-way *ANOVA*, the significance value of the variation of the treatment concentration was 0.000 or <0.05, this indicates the variation in the concentration of hand sanitizer significantly affected the formation of the inhibition zone around the well except at a concentration of 25%.

The composition of the compounds in the hand sanitizer will affect their effectiveness in inhibiting microbes. The content or composition of the compounds in each type of hand sanitizer is different. Based on table 2, it is known that there are differences in the composition of the hand sanitizer used for positive control and treatment. The Hand sanitizer control contains alcohol, glycerine, TEA, carbomer, and distilled water, while the hand sanitizer treated contains alcohol, glycerine, distilled water, and hydrogen peroxide.

Table 2. Composition comparison of the hand sanitizer control and test.

No	Composition	Hand Sanitizer	
		Control (Gel)	Test (Liquid)
1	Alcohol/Ethanol	√	√
2	Glycerin/Glycerol	√	√
3	TEA	√	-
4	Carbomer	√	-
5	Aquades	√	√
6	Hydrogen peroxide (H_2O_2)	-	√

Note = (√): exist; (-): not exist

DISCUSSION

The results showed that the hand sanitizer tested could inhibit the growth of *C. albicans* as seen from the formation of an inhibition zone in the treatment hand sanitizer. This study also shows that the higher the concentration of hand sanitizer will result in a larger diameter of the inhibition zone on *C. albicans*. This shows an increase in the effectiveness of hand sanitizers in inhibiting fungal growth along with the increase in concentration. These results are also supported by the results of statistical tests which show that there is a significant difference in the area of the inhibition zone formed in each treatment with variations in the concentration of hand sanitizers. According to Pelczar and Chan (1986) in Rini and Wijayanti (2017), the higher an antimicrobial substance or compound can cause death or inhibition of the growth of microorganisms, the faster (8). Meanwhile, according to Noer (2011) in Widyawati et al. (2017) explained that the higher the concentration of a substance, the more microorganisms that can be inhibited so that the diameter of the inhibition zone that is formed is also larger (1). The largest diameter of the inhibition zone in *C. albicans* (13.60 ± 0.55 mm) resulted in the concentration of treatment hand sanitizer 100%.

Inhibition shows the nature of disinfectants or antimicrobial substances (6). The inhibition power is divided into 4 levels, namely very strong inhibition if the diameter of the zone formed is> 20 mm, strong inhibition if the diameter of the zone is between 10-20 mm, the inhibition is sufficient (medium) if the diameter of the inhibition zone is 5-10 mm, and the inhibition is weak if

the diameter of the zone is <5 mm (6). A hand sanitizer that does not produce an inhibition zone indicates that the hand sanitizer does not have antibacterial properties against the tested germs (6). The results showed that *the hand sanitizer* tested had medium inhibition against *C. albicans* at a concentration of 100%.

Antifungal compounds have various inhibitory mechanisms against fungal cells (5). Djunaedy (2008) states that antifungal compounds have a mechanism of action by neutralizing enzymes involved in fungal invasion and colonization, damaging fungal cell membranes, inhibiting fungal enzyme systems so as to interfere with the formation of hyphal tips and affect the synthesis of nucleic acids and proteins (5).

The active ingredient in hand sanitizer alcohol-based can be ethanol (C_2H_6O), isopropanol (C_3H_8O), n-propanol, or povidone-iodine (2,3). Apart from the active ingredients, hand sanitizers generally also contain inactive components that act as thickening agents (such as polyacrylic acid), humectants (such as glycerine) or propylene glycol, and essential oils (2). The Hand sanitizer test used the active ingredient in the form of ethanol with a final concentration of about 80% (3). This concentration is in accordance with the minimum alcohol content of *hand sanitizers* recommended by the *Food and Drug Administration* (FDA) to produce a microbicidal effect, namely 60-95% (2,3).

Alcohol can kill or inactivate microbes by denaturing and coagulating proteins and dissolving lipids in cell membranes (3). This can cause cell membrane lysis (release of intracellular components) and disruption of microbial cell metabolic processes (2,3). Alcohol has broad-spectrum antimicrobial activity that effectively kills microbes in vegetative forms such as bacteria (including *multi-drug resistant Mycobacterium tuberculosis*), fungi, and encapsulated viruses such as *human immunodeficiency virus* (HIV) and *herpes simplex virus* (3,7). The drawback *hand sanitizer* or alcohol-based is that it is not effective in eliminating all types of germs such as bacterial spores, norovirus and *Clostridium difficile* (which causes diarrhea) (3).

Hydrogen peroxide (H_2O_2) plays a role in optimizing the antimicrobial effect of alcohol, where H_2O_2 is a free radical that can oxidize (damage) the components of microbial cells (3). H_2O_2 is more effective in inactivating bacterial spores than alcohol (3). Carbomer and glycerol / glycerine are used to increase the viscosity of *hand sanitizers* and act as humectants that protect skin moisture (3,7). Triethanolamine (TEA) is used to balance the pH of the *hand sanitizer* to match the skin's pH (pH 4.5-6.5) (7). Glycerine acts as an emollient gel which acts as a moisturizer which can increase the dispersibility of *hand sanitizers* as well as function as an antimicrobial (7).

CONCLUSION

The hand sanitizer tested medium inhibitory power against bacteria *C. albicans.*

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REFERENCES

1. Widyawati L, Mustariani BAA, Purmafitriah E. Formulasi Sediaan Gel Hand Sanitizer Ekstrak Etanol Daun Sirsak (Annona Muricata Linn) Sebagai Antibakteri Terhadap Staphylococcus Aureus. J Farmasetis. 2017;6(2):47–57.
2. Kumar D, Kumar S, Kumar KG, Prakash V, Prakash P, Nath G. Evaluation of the antibacterial activity of commonly used alcohol based hand sanitizers on common pathogenic bacteria. X Indian J Appl Res. 2015;3(5):2249–555.
3. Jing JLJ, Yi TP, Bose RJC, McCarthy JR, Tharmalingam N, Madheswaran T. Hand sanitizers: A review on formulation aspects, adverse effects, and regulations. Int J Environ Res Public Health. 2020;17(9).
4. Hasanah U. Mengenal Aspergillosis, Infeksi Jamur Genus Aspergillus. J Kel Sehat Sejah. 2017;15(2):76–86.
5. Yanti N, Samingan, Mudatsir. Uji Aktivitas Antifungi Ekstrak Etanol Gal Manjakani (Quercus infectoria) Terhadap Candida albicans. J Ilm Mhs Pendidik Biol. 2016;1(1):1–9.
6. Rini EP, Nugraheni ER. Uji Daya Hambat Berbagai Merek Hand Sanitizer Gel Terhadap Pertumbuhan Bakteri Escherichia coli dan Staphylococcus aureus. JPSCR J Pharm Sci Clin Res. 2018;3(1):18.
7. Asngad A, Bagas AR. Kualitas pembersih Tangan Hand Sanitizer. April Bagas R, Nopitasari. 2018;4(2):61–70.

8. Rinela A, Rini S, Wijayati N. Hand Sanitizer Ekstrak Kulit Nanas sebagai Antibakteri Staphylococcus aureus dan Escherichia coli. Indones J Chem Sci. 2017;6(1):61–6.