Synergism of Virgin Coconut Oil and Mulberry Leaves Extract as Agent in Free Alcohol Hand Sanitizer

Norhazlin Jusoh
Faculty of Applied Sciences
Universiti Teknologi Mara, Perak Branch, Tapah Campus,
35400 Tapah Road, Perak, Malaysia
norhazlin9590@uitm.edu.my

Farah Zayanah Ahmad Zulkifli
Faculty of Applied Sciences
Universiti Teknologi Mara, Perak Branch, Tapah Campus,
35400 Tapah Road, Perak, Malaysia
zayanah@uitm.edu.my

Husna Zulkipli
Faculty of Applied Sciences
Universiti Teknologi Mara, Perak Branch, Tapah Campus,
35400 Tapah Road, Perak, Malaysia
husna_zulkipli@uitm.edu.my

Emi Norzehan Mohamad Mahbob
Faculty of Applied Sciences
Universiti Teknologi Mara, Perak Branch, Tapah Campus,
35400 Tapah Road, Perak, Malaysia
emino593@uitm.edu.my

Putri Shareen Rosman
Faculty of Applied Sciences
Universiti Teknologi Mara, Perak Branch, Tapah Campus,
35400 Tapah Road, Perak, Malaysia
shareen5507@uitm.edu.my

***Abstract*— Hand hygiene is foremost techniques in preventing the spread of any health care-associated infections and keeping the health cost under control. Hand sanitizer is the key for hand hygiene when limited resources and water access. The problem is alcohol-based hand sanitizer can cause health issues such as drying skin and alcoholic poisoning. Therefore, alcohol-free hand sanitizer becomes the next approach. Investigations on hand sanitizer without alcohol for their effectiveness on microorganism"s infection are still scarce. Hence, the main objective of this study is to investigate the effectiveness of alcohol-free hand sanitizer on microbial hands carriage. The main focus is on inhibiting the growth of pure cultured bacteria from hands which were Streptococcus sp. and Staphylococcus sp. Production of alcohol-free hand sanitizer using natural products (virgin coconut oil and mulberry leaves extract) as antibacterial agents as an alternative to the current hand sanitizers in market. In the study, the antibacterial activity of alcohol-free hand sanitizer was compared with clinical hand sanitizer, commercial hand sanitizer and sterile water using disc diffusion method. Undoubtedly, clinical hand sanitizer showed the highest inhibition zone with 21 mm for both types of bacteria. On the other hand, alcohol-free hand sanitizer and commercial hand sanitizer surprisingly obtained almost similar size of inhibition zone with 14-14.5 mm. As conclusion, the alcohol-free hand sanitizer was effective in resisting the bacterial growth on hands and as promising option particularly in health impacts after use. The tested product can be introduced to selected market segments for example local pharmacies, clinics and hospitals.**

Keywords*— *hand hygiene, hand sanitizer, bacteria, antibacterial activity

I. INTRODUCTION (*HEADING 1*)

Hands are one of the main routes of transmission for microbes and any infectious agents among individuals [1]. As a personal protection, hand hygiene is foremost techniques in preventing the spread of any health care-associated infections and keeping the health cost under control [2]. Hand sanitizer is an alternative method for hand hygiene when limited resources and water access. Alcohol-based hand sanitizer is widely used especially in healthcare settings [3] but it distresses on many health issues such as drying skin, flammable and alcoholic poisoning. In addition, the alcohol hand sanitizer efficacy is reliant on amount of product is used, good practice, and usage consistency [4]. Therefore, alcohol-free hand sanitizer becomes the subsequent approach. Nevertheless, one of familiar in market in these days of alcohol-free hand sanitizer is Benzalkonium Chloride. It is comprised an active ingredient of quaternary ammonium but non-flammable, and relatively non-toxic with the low concentrations of Benzalkonium [4]. The use of chemicals as main agent in killing the microbes are still in safety concern among consumers.

There are various medicinal plants with bioactive compounds can be as antimicrobial agents and act alternative approach for antibiotic drug resistance to treat many infectious diseases. In many studies, Mulberry leaves (Morus alba) [5, 6] and virgin coconut oil (VCO) [7] are highly potential in contributing as antimicrobial agent by giving wide bioactivity spectrums against infectious organisms through multiple applications such as cosmetics, pharmaceutical and hygiene. Therefore, reliability of synergism between Mulberry leaves and VCO against infectious microorganisms in alcohol-free hand sanitizer is still not yet produced and investigated. The effectiveness of this alcohol-free hand sanitizer was then investigated by comparing with other types of hand sanitizers.

II. MATERIALS AND METHODS

A. *Pure bacteria culture*

The present study was conducted at Laboratory in Faculty of Applied Sciences, Tapah Road, Tapah, Perak, Malaysia.. Bacteria from hands were cultured directly from bare hands of two persons onto nutrient agar (NA). The NA plates were incubated in incubator 35oC and observed after 24 hours. All different types of colonies appeared were stained by using Gram staining to identify bacteria type. A total of four subcultures of bacteria were conducted and stained to get pure bacteria. The pure bacteria were clarified after obtaining similar types of bacteria for every subculture. Streptococcus sp. and Staphylococcus sp. were obtained from the 2 different individuals.

B. *Extraction of mulberry leaves*

Fresh mulberry leaves were collected in Tapah Road, Perak were picked, washed and dried in an oven at 50oC. The dried leaves were ground by using mortar pestle. A total of 15.05 g of dried mulberry leaves powder was weighed and soaked in 150 ml of 95% ethanol for 24 hours to acquire the extract. The homogenous extract solution was filtered by using the vacuum pump to get the pure solution of mulberry without impurities. The filtered solution was put into the sonicator to agitate particles in a sample. The rotary evaporator was used to distil the sample solution at 60°C. Therefore, the pure extraction of mulberry leaves was obtained in which the colour of solution was darker than the original colour.

C. *Mixture of alcohol-free hand sanitizer*

All these procedures were conducted in the biosafety cabinet to prevent any contamination. Sterile water was put into a beaker, followed by glycerine, solubilizer, and virgin coconut oil. The solution was stirred until became as homogenous mixture. Then, emulsifier was added to the solution and mixed thoroughly. Extract mulberry leaves was then added into the solution and mixed until completely homogeneous. The essential oil was lastly added with three drops while stirring it continuously. After, the solution of alcohol-free hand sanitizer was completely mixed, it was poured into universal bottle and kept it at room temperature.

D. *Antibacterial activity by disk diffusion method.*

Disk agar diffusion technique described was used for the evaluation of antimicrobial efficacy of hand sanitizers. The disk used in this method from Whatman filter paper that was cut into round shape with 6mm diameter. The disk papers were autoclaved before continuing for antibacterial activity. The two strains of pure bacteria obtained (Streptococcus sp. and Staphylococcus sp.) were swabbed entirely onto all NA plates surfaces. After that, the sterile disc papers were dipped into each solution of hand sanitizer including sterile water separately for 2 minutes before putting onto NA plates with pure bacteria. Every NA plate were divided into 4 divisions and every solution tested were conducted in duplicates. All NA plates were incubated for overnight in incubator at 35^0 C. On the next day, inhibition zones of each tested solution of NA plates were observed and measured by using a ruler. All the data were recorded in mm. The alcohol-free hand sanitizer, clinical hand sanitizer, commercial hand sanitizer (Dettol) and sterile water were compared by disk diffusion method against the pure culture of Streptococcus sp. and Staphylococcus sp. The area used for conducting all the experiments were ensured near to the flame of Bunsen burner to reduce any contamination.

III. RESULTS AND FINDINGS

The diameter sizes of the inhibition zones were measured and compared between all the solution samples against the pure culture of Streptococcus sp. and Staphylococcus sp. (Figure 1). The solutions were alcohol-free hand sanitizer, clinical hand sanitizer, commercial hand sanitizer and sterile water. Clinical hand sanitizer showed the highest inhibition zone because it contains high volume of alcohol derivatives such as ethanol, n-propanol and isopropanol with typically act as antimicrobial effective above than 70% from total volume. The rub of alcohol hand sanitizer onto skin is completely evaporated, regularly requires only 15 to 30 seconds [4]. Though, the alcohol itself flammable can cause skin drying and not suitable for long usage. Alcohol based hand sanitizers were found still not effective to destroy some species of microorganism, as an example is E.coli. Alcohol-free hand sanitizer is potentially effective as commercial hand sanitizer proven by showing almost similar size of inhibition zone for both strains of bacteria. Commercial hand sanitizer is generally alcohol–based, whereas our hand sanitizer was made from non-alcohol based (glycerine) and other natural ingredients that is safe to be used. Glycerine is a simple polyol compound that can use as a gel-based and humectant in hand sanitizer as long as moisturize the skin without any irritation. Mulberry leaves extract was found to be the best antimicrobial properties against most bacteria and fungus [8, 9]. The VCO is also fruitful in alcohol-free hand sanitizer because many studies found its antibacterial properties against most of bacteria strains including Streptococcus sp. and useful for skin treatment. The potency of VCO as antimicrobial agent is due to lauric acid with acidic pH nature [7]. In addition, the use of lemon essential oil was astringent and yet to be well known as antibacterial properties. All of these natural ingredients were very good in attributing alcohol-free hand sanitizer that defeated commercial hand sanitizer as already favoured in market. In fact, some studies on commercial hand sanitizer have observed an apparent increase in the concentration of bacteria in handprints impressed on agar plates after using [10].

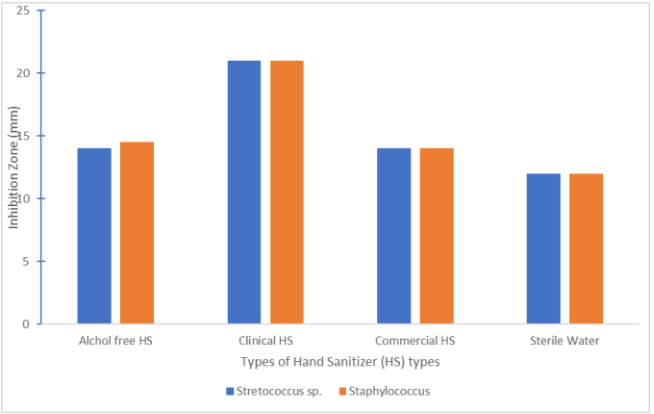

Figure 1: Comparison of different hand sanitizer (HS) including alcohol free hand sanitizer against Streptococcus sp. and Staphylococcus sp. by antibacterial activity.

IV. CONCLUSIONS

As a conclusion, the combination of mulberry leaves extract and VCO in alcohol-free hand sanitizer was effective in resisting the bacterial growth on hands and as a promising option to avoid any harm particularly health impacts due to alcohol application. This alcohol-free hand sanitizer is natural, biodegradable, non-flammable and potentially not harmful such as skin drying and alcohol poisoning. Further studies will be suggested to prove more on the side effect and with more strains of bacteria testing.

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