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Effect of *Piper Betle* extract hand sanitizer in reducing microbes on hands by using Fingertips Touch Plate Method

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Abstract: This study was performed to evaluate the effectiveness of *Piper betle* extract in hand sanitizer to reduce and kill microbes on hands compared to ordinary ingredients in hand sanitizer. *P. betle* hand sanitizers were prepared by adding 3ml of *P. betle* extract into both 3ml of ethanol and 3ml of benzalkonium chloride (BKC). Five different mixtures of hand sanitizers, *P. betle* hand sanitizer (PBHS), *P. betle* with 70% ethanol hand sanitizer (PB-EHS), *P. betle* with 0.2% BKC hand sanitizer (PB-BKCHS), 70% ethanol hand sanitizer (EHS), and 0.2% BKC hand sanitizer (BKCHS) were used to study the effect on *P. betle* as ingredient in hand sanitizer to eliminate microbes on hands. The presence of any microbes on hands were tested by fingertips touch plate method where fingertips were touched on nutrient agar plates after applying those hand sanitizers. The observation of bacterial colonies was obtained after three days. The total number, mean and percentage of bacterial colonies were calculated to compare the differences between five different types of hand sanitizers. The results showed a significant reduction in total bacterial colony counts of microbes on hands after three days of using different types of hand sanitizers. The most effective hand sanitizers, PB-EHS and PB-BKCHS did not showed any bacterial colony while PBHS, EHS and BKCHS showed 26, 7 and 3 bacterial colonies respectively. In conclusion, PB-EHS and PB-BKCHS were able to reduce microbes on hands as the total bacterial colonies on nutrient agar plates shown a significant reduction when applying these two types of hand sanitizers.

Keywords: *Piper Betle*, Daun Sirih, Ethanol, Benzalkonium Chloride, Hand Sanitizer, Fingertips Touch Plate Method

1. Introduction

This study was performed to investigate the effectiveness of *Piper betle* as active ingredient in hand sanitizer to decrease the infectious agents on hands. The infectious agents such as virus and bacteria can easily spread through contact with the infected person or touching the contaminated objects.

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Nowadays, humans are exposed to the unhealthy atmosphere. Contaminated hands can spread multiple infectious diseases from one person to another. Gastrointestinal infections, such as Salmonella, and respiratory infections such as influenza are included in these diseases. Washing hands properly and regularly will help prevent the spread of the germs that cause these diseases such as bacteria and viruses. Some types of gastrointestinal and respiratory infections, particularly in young children, the elderly, or those with a compromised immune system, may cause serious complications.

The environment that is constantly exposed to microorganisms has caused the demand and consumption of hand sanitizers increased as hand sanitizers plays an important role in today's unhealthy atmosphere. This is because when people sneeze or touch the contaminant surface, it is much difficult for them to wash their hand with soap and water, especially when they are outdoors or in the car. According to estimates by the Centers for Disease Control and Prevention (CDC), up to 80% of all infections are transmitted by hands [1]. Hand sanitizer is much more convenient as people can use it anytime, they want to clean their hands, which can avoid them getting sick and spread germs to others and that is better than not cleaning at all. People can keep a small bottle of hand sanitizer with them as their personal hygiene even when there is no soap and water especially when they are in works, schools, public transportation services and even when travelling.

Most hand sanitizers on the market are alcohol based which containing 60% to 95% of ethanol or isopropanol as it is effective concentration to kill germs. Alcohol hand sanitizer efficacy is dependent upon which and how much product is used, proper technique, and consistency of use [2]. Besides, there is also alcohol-free hand sanitizer products available today. It is containing Quaternary Ammonium Compounds (QUATS) which the most common ones used are benzalkonium chloride (BKC). The low concentrations of BKC make it relatively non-toxic and non-flammable. They pose much less of a threat in cases of accidental ingestion or as a potential fire hazard and are non-damaging to surfaces [3]. Typically, BKC are much easier on hands and continue to provide protection well after the solution has dried. These can reduce microbes however they are less effective than alcohol [1].

A natural substance which is *Piper betle* or also known as daun sirih in Malaysia, contains antiseptic and anti-fungal properties as they are rich in polyphenols especially chavicol offering dual protection from germs [4]. *P. betle* which belongs to the Piperaceae family is known for their traditional treatments to helps the wound healing process because it has a very high antioxidant content, which has ability to withstand oxidative stress and can help the wound heal faster. Furthermore, *P. betle* also used to overcome a sore throat, overcome nosebleeds, inhibit dental caries, etc [5]. Thus, in this study, *P. betle* extract were tested to identify its efficacy as active ingredient in hand sanitizer to eliminate microbes on hands using fingertips touch plate method. Therefore, the objective of this study was to investigate the effectiveness of *P. betle* as active ingredient in hand sanitizer to reduce the amount of microbes on hands using fingertips touch plate method, to find out the percentage of effectiveness of the hand sanitizers and to compare the pH value of hand sanitizer obtained with standard pH value of hand sanitizer.

2. Materials and Methods

The materials needed in this study were *P. betle* leaves, pure water, benzalkonium chloride-based hand sanitizer, ethanol-based hand sanitizer, and nutrient agar plates with composition of peptone, meat extract and agar-agar.

Perfect leaves with no damage of *P. betle* were collected and washed to eliminate impurities. Next, the leaves were soaked in the water to maintain the freshness of the leaves. *P. betle* leaves were washed thoroughly in tap water and dried in oven at 100˚C for 15 minutes. Then, *P. betle* leaves were crushed into pieces by using blender. 5 gram of *P. betle* leaves were weighted using an electric balance and boiled with 100mL of pure water on a hot plate at 100˚C for 15 minutes. The extract was filtered with filter paper until the clear water extract obtained.

All equipment used were autoclaved to ensure that no external bacteria will interfere this test. The hand sanitizer solutions were prepared in laminar flow. For the first hand sanitizer solution, 3 mL of *P. betle* extract was added into 3 mL of ethanol hand sanitizer. For the second solution, 3 mL of *P. betle* extract was added into 3 mL benzalkonium chloride hand sanitizer. The third hand sanitizer was *P. betle* extract. The fourth and fifth hand sanitizers were 70% ethanol hand sanitizer and 0.2% benzalkonium chloride hand sanitizer. The pH of PBHS, PB-EHS, PB-BKCHS, EHS and BKCHS were taken using pH meter and the readings were recorded.

The antiseptic tests of hand sanitizer involved three students. All respondents were required to do the same activities without washing their hands such as open the doors, using phones, using computers at the café cyber and taking a bus to the campus. Then, all respondents were required to make a fingerprint on nutrient agar plate without washing hands or using any hand sanitizer after the activity done before. Plates with fingerprints were incubate at 37˚C for 72 hours. After incubation, the number of colonies were counted.

The respondents were required to use hand sanitizer which each person need to use five types of hand sanitizer which are PBHS, PB-EHS, PH-BKCHS, EHS and BKCHS. They required to use two drops of hand sanitizer then apply it evenly on their finger and let stand for one minute. Next, the respondents were required to make a print of their fingertip on the nutrient agar plates. The same step were repeated using other hand sanitizers on different fingertips. Next, the nutrient agar plates were incubated at 37˚C for 72 hours. After incubation, the number of colonies were counted.

The data were presented using bar chart. The total number of bacterial colonies were counted for different types of hand sanitizers after three days. The mean of bacterial colonies was also calculated for different types of hand sanitizers. Besides, the percentage of effectiveness of different hand sanitizers were also calculated using the equation below:

$$\text{Effectiveness} = \frac{A-B}{A} \times 100\% \qquad \textbf{Eq. 1}$$

where A is total number of for control and B is total number bacteria colonies for each hand sanitizer.

3. Results and Discussion

Result and discussion of this study are shown as below.

3.1 Results

Table 1: The number of bacterial colonies obtained before and after applying hand sanitizers

Hand sanitizers	Total number of bacterial colonies before applying hand sanitizer (Control)	Number of bacterial colonies after applying hand sanitizer		
		Student 1	Student 2	Student 3
PBHS	187	12	6	8
PB-EHS	187	0	0	0
PB-BKCHS	187	0	0	0
EHS	187	3	3	1
BKCHS	187	2	1	0

The study showed a significant reduction in total bacterial colony counts of microbes on hands using PB-EHS and PB-BKCHS as compared to PBHS, EHS and BKCHS. From **Table 1**, the number of bacterial colonies before applying were 187 and the number were reduced when the respondents applying different types of hand sanitizers.

Table 2: Result of bacterial colonies obtained after three days using different types of hand sanitizers

Hand sanitizers	Total number of bacterial colonies	Mean	Effectiveness (%)	pH
PBHS	26	8.67	86.09	5.6
PB-EHS	0	0	100	6.0
PB-BKCHS	0	0	100	6.4
EHS	7	2.33	96.26	7.1
BKCHS	3	1	98.40	7.3

The result of bacterial colonies obtained after three days using different types of hand santizers were shown in **Table 2**. Between five different hand sanitizer that we used, three of them showed the growth of bacterial colonies while the other two hand sanitizer did not show any bacteria growth. The highest total number of bacterial colonies presented using the PBHS is 26, followed by EHS and BKCHS which are 7 and 3.

Figure 1 illustrates the bar graph of the total colony counts for PBHS, PB-EHS, PB-BKCHS, EHS and BKCHS after three days. The graph demonstrates that PB-EHS and PB-BKCHS had less bacteria than other hand sanitizers. Based on **Figure 1**, PBHS, EHS and BKCHS showed the growth of bacteria colonies while the PB-EHS and PB-BKCHS did not showed any growth of bacteria colonies. PBHS has the highest number of bacteria colonies which is 26 compared to EHS and BKCHS that have the smaller number of bacteria colonies which are 7 and 3. The presence of *P. betle* extract in EHS and BKCHS showed that they were most effective hand sanitizers in killing bacteria that present on the skin than EHS and BKCHS alone.

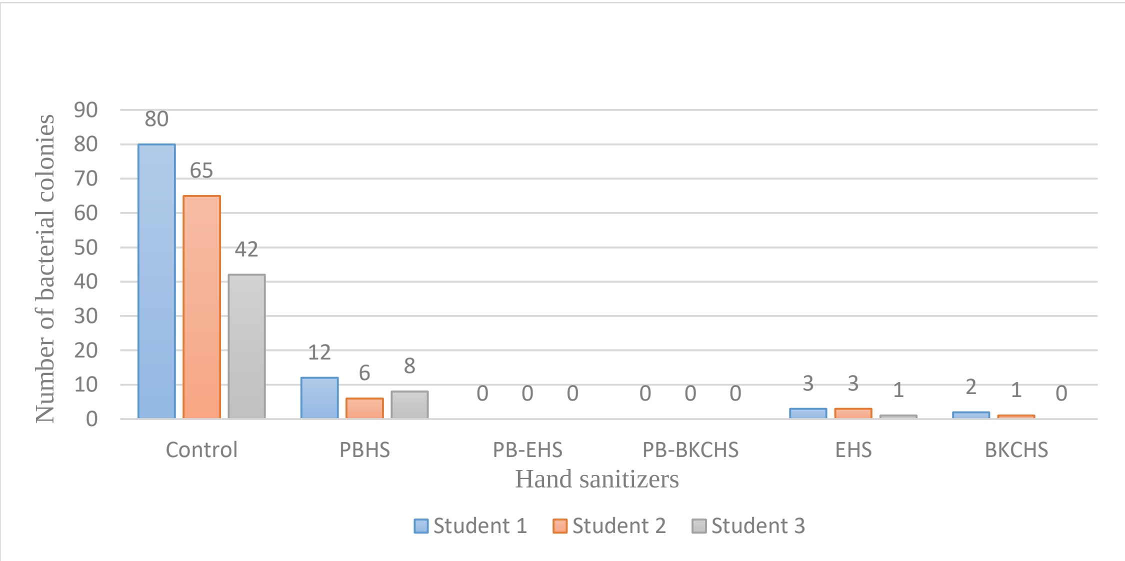

Figure 1: Number of bacterial colonies after three days for different types of hand sanitizers

The mean of colony count for PBHS after 3 days was 8.67, for EHS was 2.33, BKCHS was 1 while for both PB-EHS and PB-BKCHS are 0 as shown in **Figure 2**. On average, PB-EHS and PB-BKCHS eliminates 100% bacteria as they did not show any bacterial growth on nutrient agar plates followed by BKCHS with 98.4% effectiveness. EHS shows 96.26% effectiveness in killing bacteria while PBHS shows the lowest effectiveness of 86.09%. Based on the **Figure 2**, the mean of bacterial colonies for BKCHS is 1 and the mean of bacterial colonies for PBHS is 8.67, while 2.33 is the mean of bacterial colonies for EHS. PB-EHS and PB-BKCHS has 0 mean as they did not show any bacterial growth on nutrient agar plates.

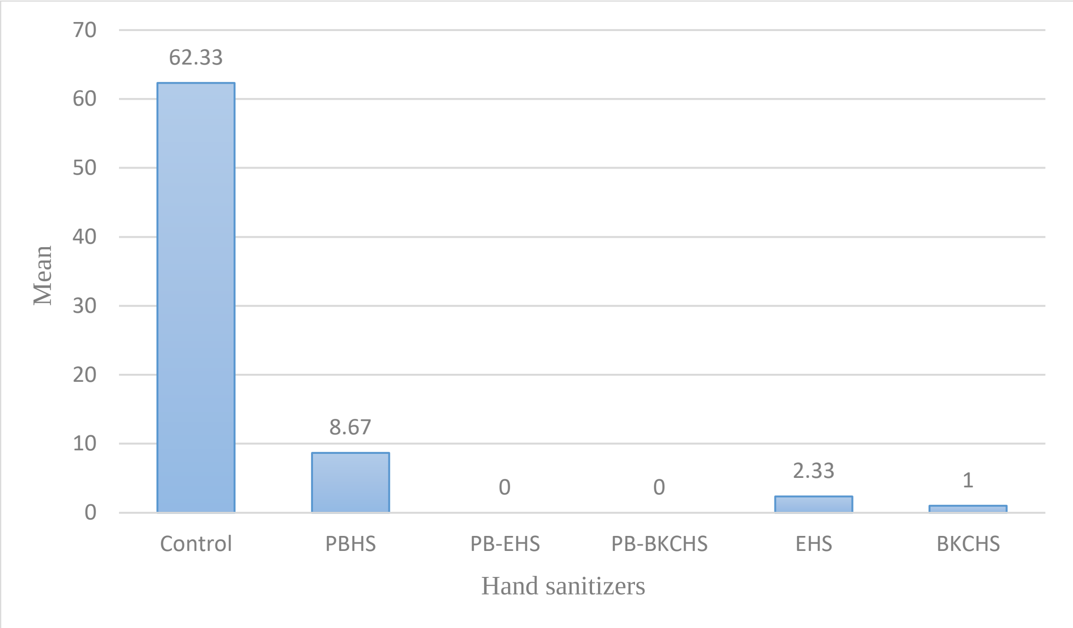

Figure 2: Mean of bacterial colonies for different types of hand sanitizers after three days

From **Figure 3**, the pH analysis, PBHS has pH of 5.6 while pH for PB-EHS is 6.0 and pH for PB-BKCHS is 6.4. These three hand sanitizers were more acidic than EHS and BKCHS which each of them has pH of 7.1 and 7.3. Based on Figure 3, PB-EHS and PB-BKCHS showed the most effective hand sanitizers in killing microbes on hands as they have 100% potential to eliminate bacteria. EHS and BKCHS also have a great potential in killing bacteria as their effectiveness were above than 90%. PBHS showed the lowest percentage than other hand sanitizers with 86.09% effectiveness.

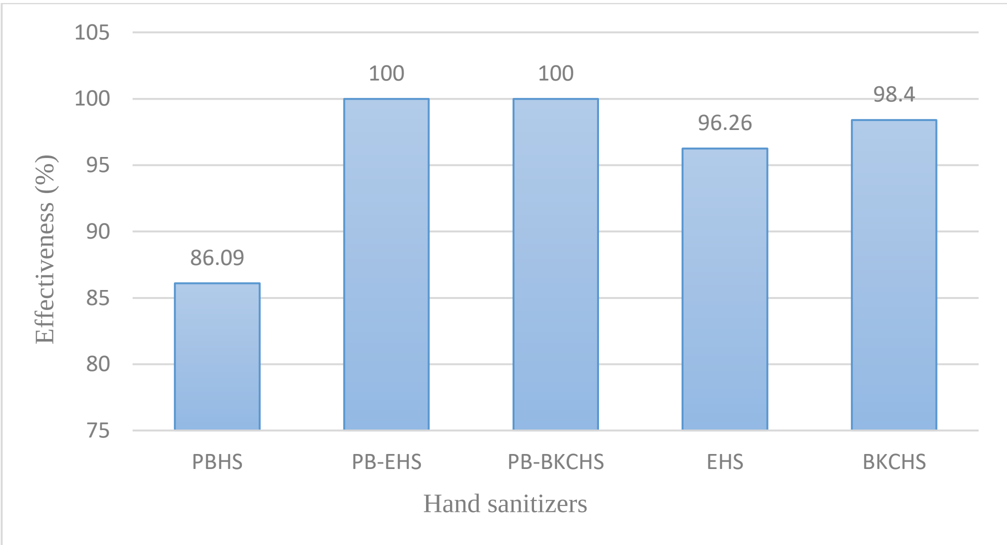

Figure 3: Effectiveness of different types of hand sanitizers

3.2 Discussions

PB-EHS and PB-BKCHS were more effective than hand sanitizer that based of *P. betle*, ethanol and BKC alone. Although PBHS can reduce the growth of bacterial colonies but with the presence of ethanol and BKC in *P. betle* hand sanitizer, the result became more effective with no growth of bacterial colonies on agar plates. Using PB-EHS and PB-BKCHS were considering that bacteria hard to grow, non-toxic and have a good absorbance. EHS and BKCHS were used as a benchmark to test whether PB-EHS and PB-BKCHS were effective in disinfecting germs as EHS and BKCHS have been proven clinically to be effective in killing germs. For pH analysis, PBHS, PB-EHS and PB-BKCHS followed the standard pH of hand sanitizer which conform to the skin's pH of 4.5 to 6.5. The pH value of the hand sanitizer that does not conform to the pH of the skin will cause irritation to the skin. The topical dose should be 4.5 to 6.5 in the pH range of the skin. If it is too acidic, it may cause irritation to the skin, and if it is too alkaline, it may cause scaly skin. This occurs because the acidic mantle on the stratum corneum layer of the skin has been damaged [6].

3.3 Limitations

The limitations to our study were that the study population was small at three test subjects, there was no attempt to observe or document compliance with hand hygiene protocols, and the study was limited to evaluation of only one pathogenic bacteria species.

4. Conclusion

The results of this study suggested that, *P. betle* with 70% EHS and *P. betle* with 0.2% BKCHS were able to reduce bacteria as the total bacterial colonies on nutrient agar plates shown a significant reduction when applying these two types of hand sanitizers. This finding suggested that *P. betle* has shown its effectiveness in reducing bacteria as it has anti-bacteria properties. The presence of *P. betle* in ethanol and BKC as active ingredient has increased the effectiveness of both hand sanitizers. Ethanol and BKC are compulsory ingredients in this study as these two components were complying the current guidelines for hand hygiene as recommended by Disease Control and Prevention (CDC). *P. betle* with EHS and *P. betle* with BKCHS followed the standard pH of hand sanitizer which conform to the skin's pH of 4.5 to 6.5. The pH value of the hand sanitizer that does not conform to the pH of the skin will cause irritation to the skin. The topical dose should be 4.5 to 6.5 in the pH range of the skin. If it is too acidic, it may cause irritation to the skin, and if it is too alkaline, it may cause scaly skin. Thus, the results of this study demonstrate and showed the great potential of *P. betle* as an active ingredient in reducing microbes on hands.

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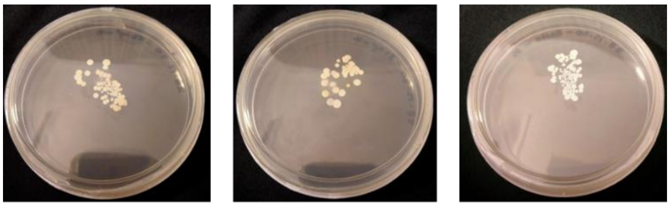
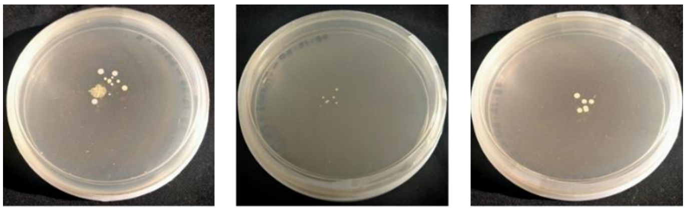
Appendix A

Figures below show the presence of colony on nutrient agar plates.



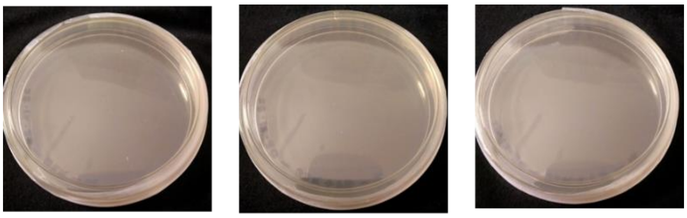
Student 1 Student 2 Student 3

Figure 4: Number of bacteria colonies for control



Student 1 Student 2 Student 3

Figure 5: Number of bacteria colonies for PBHS



Student 1 Student 2 Student 3

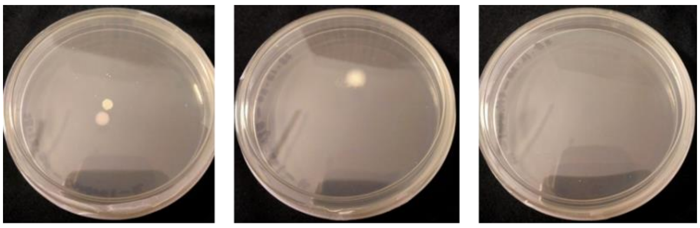
Figure 6: Number of bacteria colonies for PB-EHS

Student 1 Student 2 Student 3

Figure 7: Number of bacteria colonies for PB-BKCHS

Student 1 Student 2 Student 3

Figure 8: Number of bacteria colonies for EHS



Student 1 Student 2 Student 3

Figure 9: Number of bacteria colonies for BKCHS

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