

Antimicrobial Evaluation of *Piper betel* Leaves and Lime Based Hand Sanitizer

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ABSTRACT

Background: The needs of hand sanitizer increase significantly during the COVID-19 outbreak. **Objectives:** In this study, we prepared *Piper betel* and lime based hand sanitizer with a simple maceration technique. **Materials and Methods:** After soaking *Piper betel* in the hot water for 24 h, the water was collected and lime juice was added into the mixture to prepare samples with concentrations of 20, 40, 60 and 80%. **Results:** The anti-bacterial activity test showed that sample of 60% exhibited the best result among other samples with pH of 4.1. **Conclusion:** From this study, the sample of 60% was promising to be developed as hand sanitizer. **Key words:** Hand sanitizer, Maceration, *Piper betel*, Lime juice, Anti-bacterial.

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INTRODUCTION

Since the outbreak of COVID-19, hand sanitizer is highly needed for reducing our risk of catching the infection. Hand sanitizer is an important product in this outbreak, which has many types such as liquid, gel, or cream. Our hands may be contaminated by touching some surfaces with patients' respiratory droplets from a sneeze or cough which may then facilitate the transmission of the disease.^[1-3]

Almost all hand sanitizer products in the market are made of alcohol. Ethanol is the most popular alcohol as raw material of the product which is usually used to make a concentration of 50 to 70 %.^[4,5] Alcohol has anti-bacterial activity for many kinds of bacterial. However, alcohol has drawbacks because it is flammable and makes the skin of the hands dehydrated.

Recently, many people have been using herbal based products i.e. hand sanitizer. *Piper betel* is one of the most famous plants which is commonly used as an antiseptic and mouth freshener.^[6] A commercial tooth-paste with a *Piper betel* leaf extract is also available in the market due to its antiseptic function. *Piper betel* is the leaf of a vine belonging to the Piperaceae family, which has silky heart shaped leaves and white catkin.^[7] The *Piper betel* plant is abundantly found in south and Southeast Asia such as India, Indonesia, Malaysia, Thailand, Bangladesh etc. Many people use *Piper betel* leaves traditionally by boiling it in the water. After cooling, the water is used as mouthwash or bodywash. *Piper betel* leaf has special smell and has essential oil about 4.2%. The essential oil has phenol as main compound. This phenolic compounds are bethelphenol and chavicol. Chavicol is aromatic compound which is a source of *Piper betel* special smell. There are some

derivatives of the compounds such as chavibetol, carvacrol, eugenol, allipyrocatechol and catechin. A phenolic compound in *Piper betel* essential oil has anti-microbial and anti-fungal properties. That is why the *Piper betel* is able to inhibit the growth of bacteria. Similarly, lime has flavonoid compound which contributes to its antioxidant and anti-bacterial properties. Several studies showed that lime juice has anti-viral and anti-fungals properties.^[8,9] It is very interesting to investigate *Piper betel* and lime as raw materials of hand sanitizer.

MATERIALS AND METHODS

Preparation of samples

Piper betel leaves about 50 g as main materials were washed using tap water and then were cut in small pieces. The cut leaves were dried in hot air for 15 h. After drying, the leaves were soaked in the hot water for 24 h. The water was separated and was added by 8 ml lime extracts. Distilled water was added into the mixture to make samples with concentrations of 20, 40, 60 and 80%.

pH evaluation

The acidity of the samples was checked by using a digital pH meter. The small amount of samples was loaded into the tubes. The electrode was soaked into the tubes to measure the pH of the samples.

Disc diffusion method

Firstly, the antibacterial activities of samples were evaluated using disc diffusion method. 15 ml of molten nutrient agar media was poured into sterile

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petriplates to prepare the agar plates. After a few minutes, 0.1 % inoculum suspension was swabbed uniformly on the plates and was allowed to stand for 15 min.

The different dilutions of mixture (20, 40, 60 and 80%) were loaded on 5 mm autoclaved filter paper discs. The loaded discs were placed on the surface of the media and the plates were incubated at 37°C for 24 h. Finally, the inhibition zones formed around the disc were measured by a ruler in millimeter.

Hand sanitizer application

The best sample from the disc diffusion method was used in the hand sanitizer application. In order to compare the performance, commercial hand soap and alcohol-based hand sanitizer were used. Breads as the media were touched using bare hands with different treatments such as dirty hands, hand soap, alcohol-based handsanitizer and the best sample. After 10 days, the breads were checked to investigated the microbial growth.

RESULTS

The acidity of samples was checked using digital pH meter and as displayed in Figure 1. The higher concentration decreased the pH value of samples from 7.6 to 3.8. This is because citric acid of lime juice reduced the pH value of the samples. Disc diffusion tests showed that the sample of 60% was the most effective in inhibiting the growth of bacterial as displayed in Figure 2. The sample has a transparent area indicating the inhibition area that was 19.3 mm in diameter as measured by a ruler. Other samples did not show a significant inhibition of bacterial growth. The sample of 60% was tested as hand sanitizer and was compared with hand soap, alcohol-based hand sanitizer and non-treatment hands. Then, the hands were swabbed onto breads as microbial growth medium. Figure 3 shows the difference microbial growth on breads after 10 days. Bread swabbed by non-treatment hands showed the largest area of microbial growth as shown by black and brown area on the bread. The breads of alcohol-based hand sanitizer and hand soap exhibited the a small area of dark spots indicating the microbial growth. The sample of 60% successfully inhibited the growth by showing no dark spot on the bread.

DISCUSSION

The sample of 60% showed the most effective anti-microbial among other samples. The pH value was 4.1 because the citric acid from lime juice increased the acidity of the sample. Phenolic compounds of *Piper betel* leaves were able to inhibit the microbial growth. The lime juice also

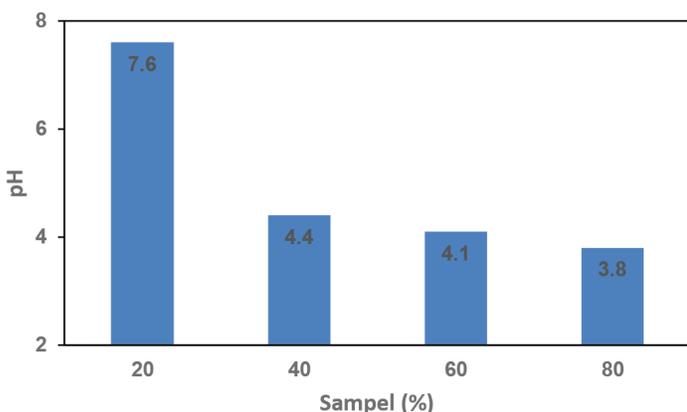


Figure 1: The pH value of samples with concentration of 20, 40, 60 and 80%.

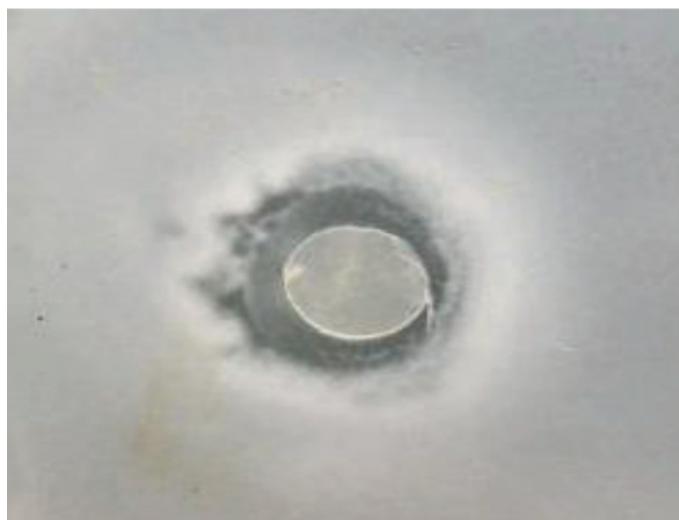


Figure 2: The disc diffusion test of the sample of 60%.

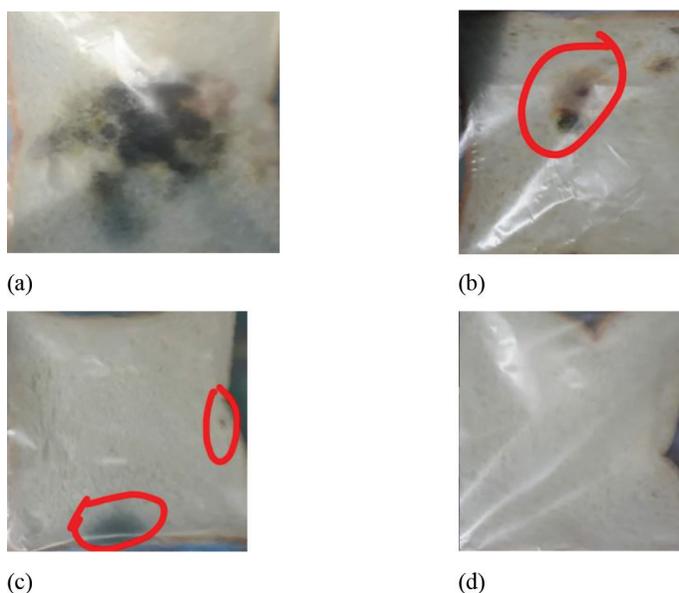


Figure 3: Swab test using bread as media for non-treatment hands (a), alcohol based hand sanitizer (b), hand soap (c) and the sample of 60% (d).

contributed in the antimicrobial activities which is similar to other results.^[10] Citric acid, the major organic acid of limejuice played important role in inhibiting the microbial growth. There are two microbes habitation on hands named resident and transient floras.^[11] The resident floras colonize the deeper skin layers and are resistant to mechanical removal.^[11] The transient floras such as *E. coli*, *S. aureus* and *Pseudomonas aeruginosa* colonize the superficial layer skin in a short period of time.^[11] The hand soap and alcohol based results were less effective compared to the sample of 60%. This might be caused by microbes from deeper skin or resident floras have strongly attached to hands. The phenolic compounds and citric acid in the sample might deeply removed microbes both resident and transient floras.

CONCLUSION

The sample of 60% showed the most effective among other samples in inhibiting microbial growth. The pH of samples was reduced due to the

increase of citric acid. There is no dark spot indicating the microbial growth on the bread of the sample after 10 days. The results exhibited that the sample was more effective than alcohol-based hand sanitizer and hand soap in inhibiting the growth. This might be caused by the sample could penetrate deeper into the skin layers and removed both resident and transient floras. The combination of *Piper betel* leaves and lime juice is promising in developing herbal hand sanitizer in the near future.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS USED

E. coli: *Escherichia coli*; *S. aureus*: *Staphylococcus aureus*.

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GRAPHICAL ABSTRACT



SUMMARY

- Piper betel* and lime based hand sanitizer has been successfully prepared by a simple maceration technique.
- The sample with the concentration of 60% showed the best antimicrobial activities among other samples.
- Our comparison study exhibited that the sample has better antimicrobial than commercial hand soap and alcohol based hand sanitizer.
- The sample could penetrate deeper into the skin layers and removed both resident and transient floras.

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