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Bio-Hand Sanitizer Based on Peel of Lime (*Citrus aurantifolia* S) and Leaves Betel (*Piper betle* L)

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Abstract: The large number of antiseptic products on the market provide choices for people to choose alcoholic or non-alcoholic hand sanitizers. Most commercial hand sanitizers are 70% to 90% alcohol-based. For some people, the use of alcohol-based hand sanitizers is not friendly for sensitive skin. Therefore, in this study efforts were made to minimize the use of alcohol as an antiseptic agent in hand sanitizers by using lime peel extract and betel leaf extract. Betel leaves contain chemicals that are useful as antiseptic, antibacterial, and antioxidant substances. The lime peel contains flavonoid compounds that are useful as antioxidants, antiseptics, anticancer, anti-inflammatory, and antibacterial. The results showed that the extraction of lime peel was dark brown and betel leaves were blackish brown. Lime peel extraction was carried out for 5 hours using the maceration method while lime peel extraction used the reflux method. The results of the inhibition test using the Disk Diffusion Test method showed that the growth of bacteria in the sample area grew less compared to the eco-enzyme and water. While the results of the organoleptic test showed that bio-hand sanitizer products made from the lime peel and betel leaf were quite attractive to respondents.

Kata kunci: antibacterial, COVID-19, hand sanitizer, lime peel

Abstrak: *Banyaknya produk antiseptik yang beredar di pasaran memberikan pilihan bagi masyarakat untuk memilih hand sanitizer beralkohol atau non alkohol. Sebagian besar pembersih tangan komersial berbahan dasar alkohol 70% hingga 90%. Bagi sebagian orang, penggunaan hand sanitizer berbahan dasar alkohol tidak ramah untuk kulit sensitif. Oleh karena itu, dalam penelitian ini dilakukan upaya untuk meminimalkan penggunaan alkohol sebagai zat antiseptic pada handsanitizer dengan menggunakan ekstrak kulit jeruk nipis serta ekstrak daun sirih. Daun sirih mengandung bahan kimia yang berguna untuk zat antiseptik, antibakteri, dan antioksidan. Kulit jeruk nipis mengandung senyawa flavonoid yang bermanfaat sebagai antioksidan, antiseptik, antikanker, antiradang, dan antibakteri. Hasil penelitian menunjukkan hasil ekstraksi kulit jeruk nipis berwarna coklat tua dan daun sirih berwarna coklat kehitaman. Ekstraksi kulit jeruk nipis dilakukan selama 5 jam menggunakan metode maserasi sedangkan ekstraksi kulit jeruk nipis menggunakan metode refluks. Hasil uji daya hambat menggunakan metode Disk Diffusion Test menunjukkan pertumbuhan bakteri di daerah sampel tumbuh lebih sedikit dibandingkan dengan eko-enzim dan air. Sedangkan hasil dari pengujian organoleptik bahwa produk bio-hand sanitizer berbahan dasar kulit jeruk nipis dan daun sirih cukup diminati responden*

Keywords: *antibakteri, COVID-19, handsanitizer, kulit jeruk nipis*

INTRODUCTION

The COVID-19 pandemic has had a significant impact on public awareness of health. The spread of the virus, which did not subside until entering its second year in Indonesia, has resulted in a high and stable need for practical hand sanitizers. Because hand sanitizers are believed to be able to kill bacteria and viruses that cause COVID-19. The number of antiseptic products on the market gives people the choice to choose alcoholic or non-alcoholic hand sanitizers. However, most commercialized hand sanitizers are alcohol-based 70-90%. Some of the side effects of using alcohol-based hand sanitizers cause skin biome (allergy), dryness, and skin dehydration (Misrita *et al*. 2020). In Indonesia, there are variations of natural ingredients that provide the benefits of killing bacteria and viruses. One of them is lime peel extract (Citrus aurantifolia) which contains secondary metabolite compounds such as flavonoids, alkaloids, saponins, and phenolics which

are active ingredients in antiseptics (Dollu *et al*. 2020; Triyani *et.al*. 2021). In addition, natural ingredients that can be used are betel leaf (*Piper betle* L) which contains flavonoid compounds, polyphenols, tannins, and essential oils. Derivatives of these compounds have a bactericidal activity 5 times stronger than phenol. In addition to containing antiseptic substances, betel leaf can kill bacteria and fungi and has antioxidant power (Effendi *et al*. 2020; Ramadhanty & Anwari 2021).

These two basic ingredients when combined with less alcohol content will minimize the side effects of allergies and dehydration on the skin. So, it is potential to use these natural ingredients as bio hand sanitizers with other mixtures (Mayna & Fatimah 2021). This multidisciplinary project is expected to increase the value of betel leaf and orange peel, as well as broaden the horizons and skills of team members and readers in processing them into natural hand sanitizers. The aims of this research are to identify the potential of betel leaf and lime peel as an active ingredient in hand sanitizers from natural ingredients, compare the effectiveness of betel leaf and lime peel-based bio-hand sanitizers with commercial hand sanitizers, and produce hand sanitizer products based on natural ingredients to minimize alcohol content.

MATERIALS AND METHODS

Materials

Ethanol 70% commercial, Aqua DM, glycerin (96% technical) commercial, peppermint essential oil commercial, sweet orange essential oil commercial, lime peel, betel leaf, and nutrient agar from aloe vera. Instrumentation analysis from Fadlilah, *et.al.* 2010 is High-Performance Liquid Chromatography (HPLC) analysis performed using an Agilent 1200 HPLC system from Agilent (Karlsruhe, Germany), equipped with a quaternary pump, an autosampler, and a VWD UV detector. Quercetin was separated from the sample solutions using a C18 column (4.6×250 mm I.D, particle size 5 µm, Agilent Eclipse XDB- C18), with a mobile phase consisting of methanol and 0.40% phosphoric acid (49:51, V/V), at 25℃. The flow rate was 1.0 ml/min, and injections were 20 µl in volume.

Preparation for Making Bio-Hand Sanitizer

The betel leaf was washed, grated/peeled the lime peel, the lime peel and betel leaf were cut into smaller pieces, the lime peel and betel leaf were transferred to a placemat, and placed in an oven at 60°C for 4 hours, and the glycerin was mixed into a solution of ethanol and essential sweet orange oil commercial with a ratio of 1:1 into a humidifier, and the softener is stored in the refrigerator in a closed glass bottle.

Extraction of Lime Peel Using the Maceration Method

Dried lime peel (80 g) was put in a 1000 mL bottle, 800 mL of ethanol 70% w/v was added to the bottle, allowed to stand for 72 hours, filtered using a Buchner funnel and filter paper, the liquid was put in a rotary evaporator, the temperature was adjusted according to the boiling point of the solvent. (Ethanol, 70°C), The extraction results are stored in vials.

Extraction of Betel Leaf Using Reflux

Betel leaves that have been cut into small pieces are weighed 20 g and then put into a round flask containing a stirrer, added 200 mL of 70% ethanol, placed in a bath filled with water and placed on a hotplate then a condenser is installed on it, set the temperature to 70℃ bath temperature, the hotplate is turned off and wait for the solution to cool, filter the solution using a Buchner, and put it in a bottle.

The Stage of Mixing the Ingredients into a Bio-Hand Sanitizer

Prepare a 1 L beaker, add 571.4 mL of 70% ethanol (40% final concentration), 50 mL of lime peel, and betel leaf extract, add 15 mL of moisturizer (Aloe vera), add distilled water to a total volume of 1 L, stir at room temperature with a stirring rod, then, the pH is measured, the sample is ready to be put in a 100 mL spray bottle.

Antibacterial Activity Test

Four types of samples were prepared, such as betel leaf and lime peel hand sanitizer, eco-enzyme hand sanitizer, aseptic gel hand sanitizer, and aquades as a control to test the inhibition of *Escherichia coli* ATCC 25922 bacteria at Pertamina University Laboratory.

Organoleptic Test

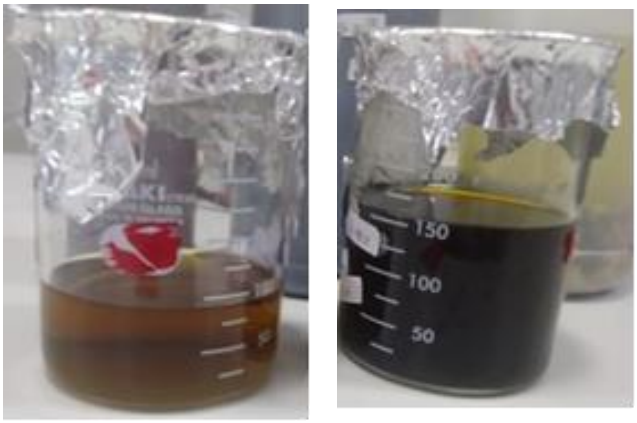
The organoleptic test refers to research by (Setyawardhani & Saputri 2021). Hand sanitizer samples were tested on 10 people (consisting of practitioners, fellow practitioners, and random practitioners)

RESULT AND DISCUSSION

Bio-hand sanitizer is a hand hygiene product made from natural ingredients made from green betel leaf extract (*Piper betle* L), lime (*Citrus aurantifolia* S) then added aloe vera in addition to functioning as an antiseptic and antibacterial. It can also be a moisturizer with efficacious properties to maintain healthy skin. These materials can be used as bio-hand sanitizers because they contain flavanol compounds (Mattila *et al*. 2000). In this research, the first step is material preparation. The materials used are lime peel and betel leaf, both materials are cleaned and cut into small pieces to facilitate the extraction process. Then lime peel and betel leaf are put in an oven at 60°C for

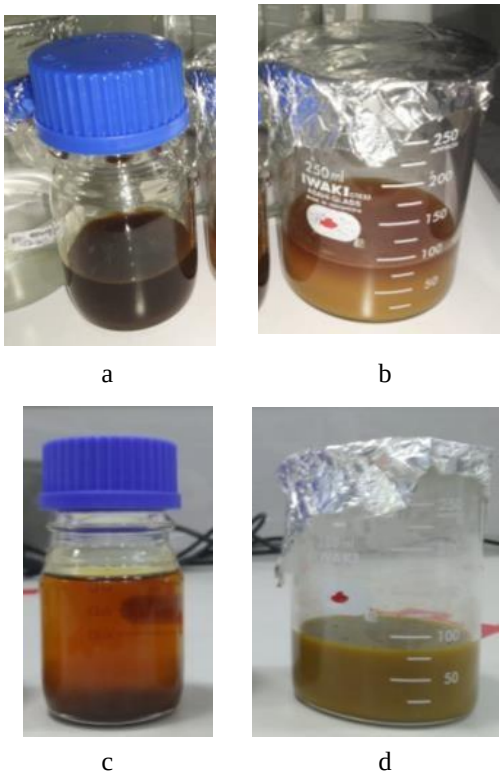
4 hours. The drying process using this oven aims to maintain the content of active compounds in lime and betel leaves, and so that sample can be stored for long time until the extraction process (Wahyuni 2014).

The extraction process of lime and betel leaf aims to separate substances from solid and liquid materials using a solvent (Fajarwati 2013). The solvent used is 70% ethanol, ethanol has a polar group (alcohol) so that it can attract polar compounds, such as flavonoid compounds. In addition, the use of ethanol as a solvent in this hand sanitizer can increase the levels of quercetin which is an important compound with antibacterial or antiseptic properties. However, before the extraction, maceration was carried out for 72 hours to obtain the initial extract without the aid of heating from a hot plate. The materials used in this study carried out a different extraction process (maceration and reflux). Lime peel uses reflux, and betel leaf uses maceration/ The following results from the extraction of lime and betel leaf can be seen in Figure 1.



a b

Figure 1. Extraction results of (a) lime peel using maceration and (b) betel leaf using reflux distillation for 5 hours



a b

c d

Figure 2. The results of the extraction of betel leaf (a) and lime peel (b) before Buchner and betel leaf (c) and lime peel (d) after Buchner

Based on the results of the study, shown in Figure 2 the extraction results of (a) lime peel using a rotary evaporator is dark brown with the smell of lime peel and (b) betel leaf using reflux distillation for 5 hours is observed to be blackish brown.

Based on the results of the study, it is shown in Figure 2 that there are significant differences in the results of the extraction of betel leaf and lime peel before and after in Buchner. In the treatment after Buchner, the color of the betel leaf (c) was clearer than before in Buchner (a) the betel leaf. Likewise, lime peel extract (d) is greenish yellow and significantly different from before in Buchner which was observed in (b) which is a faded brown color. This difference is caused by the presence of agglomerates that may still be available in the extracted solution before Buchner. And when in Buchner the agglomerates are separated so that the resulting color is clearer than before.

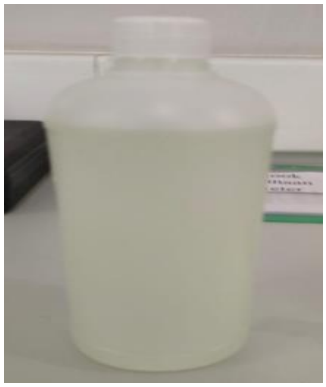


Figure 3. Bio-hand sanitizer product (the result of mixing lime peel extract, betel leaf, essential sweet orange oil commercial, peppermint, glycerin, and 40% ethanol).

Based on the results of the study, in Figure 3 the results of the mixing of the bio-hand sanitizer product, it is observed that the color is slightly yellowish and smells good which comes from a mixture of extract of lime and betel leaf. The results of the mixing of the bio-hand sanitizer products were then measured pH to determine the sensitivity of the hand sanitizer product to the skin based on SNI No. 06-2588 with a pH range of 4.5-6.5. From the results of pH measurements on bio-hand sanitizer products, pH 6.31 is obtained, this result is included in SNI No. 06-2588. The bio-hand sanitizer product is mixed with 40% ethanol, this aims to produce a hand sanitizer product that is less alcoholic. From pH measurements, it can be concluded that the bio-hand sanitizer we get is less alcoholic. However, this is not enough. Because it has to check its antibacterial activity through bacterial inhibitory testing using *E. coli* bacteria and characterization analysis using HPLC through literature studies and organoleptic testing.

The resulting bio-hand sanitizer product was then analyzed for bacterial inhibition using *E. coli* and characterization analysis using HPLC through a literature study, In the test of bacterial inhibition, two comparisons were used, as can be seen in Figure 4.

From the results of the bacterial inhibition test on the bio-hand sanitizer sample, data is generated, as shown in Figure 5.

a b

Figure 4. Hand sanitizer gel products (a) and (b) Hand Sanitizer Eco as a comparison for bio-hand sanitizers in testing bacterial inhibition.

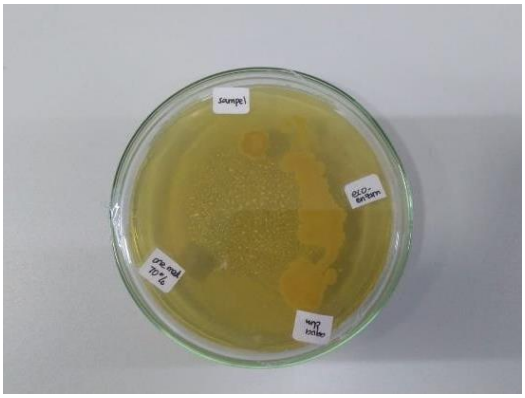


Figure 5. The results of the inhibition test using the *Disk Diffusion Test* method.

Based on the *Disk Diffusion Test*, the effectiveness of the hand sanitizer (sample) is slightly ambiguous. This happens due to the uneven growth of bacteria. However, when compared other samples such as eco-enzyme-based hand sanitizers, 70% alcohol commercial hand sanitizers, and controls (aquadest), the growth of bacteria in the sample area grew less compared to eco-enzyme and water. However, it is still proven that bacteria have not grown at all in the 70% alcohol commercial hand sanitizer area. For the measurement of the inhibition

zone of the sample, it cannot be measured because the growth results are attached and *E. coli* bacteria grow on paper that is given hand sanitizer. This is because when the incubation is carried out for too long, over time more cells will grow so that the nutrients will run out and over time the nutrients will die. Another factor is that the incubation period when using *E. coli* is 1×24 to 2×24 hours.

To support the results of the analysis, a literature study was conducted regarding the identification of flavanol compounds (Quercetin) as antibacterial compounds through HPLC analysis reported by several researchers, as reported by (Fadlilah, *et.al*. 2010) that the compound inhibiting *E. coli* bacteria is quercetin, which is a type of flavonoid flavanol. The mechanism of flavonoids used as antibacterial is the process of denaturing bacterial cells and disrupting the work of the membrane. The process of protein denaturation results in the coagulation of bacterial cell wall proteins. If bacteria do not have a cell wall, they cannot withstand external influences and soon die. The results of the HPLC analysis can be seen in Figure 6.

Figure 6 is the standard content of quercetin which was analyzed using HPLC. It can be seen that the retention time of 2.5 min is a quercetin compound with an area of of 98.7420%. In a study conducted by Yuni (2020) lime can inhibit the growth of *E. coli* well at a concentration level of 40%. His research also states that the higher the concentration used, the better the inhibition will be. It was also reported by Malik (2019) that antimicrobial testing of betel leaf methanol extract using the Disk diffusion method obtained negative results against gram-negative bacteria *E. coli* with concentrations reaching 120 ppm. A study conducted by Mahyuni. (2016) explained that lime peel has the highest polyphenol content reaching 6.954 mg/g compared to leaves and contents as shown in Table 1.

From Table 1, it can be seen that the fruit peels have high levels of quercetin, this is based on the total content of dissolved polyphenols in the lime peel extract. It was also shown in the HPLC chromatogram results that the quercetin content of the fruit peel was higher than other compounds (Mahyuni 2016; Cahyono *et al*. 2021). Purba *et.al*. (2019), reported that the quercetin content of betel leaf extract in ethanol solvent produced through HPLC analysis as shown in Figure 6, was observed at a retention time of around 27.82 with a wavelength of 272 nm it produces a percent absorbance of about 125 mAU and is the second highest level after eugenol which has a percent absorbance of 132 mAu with a retention time of 40 min.

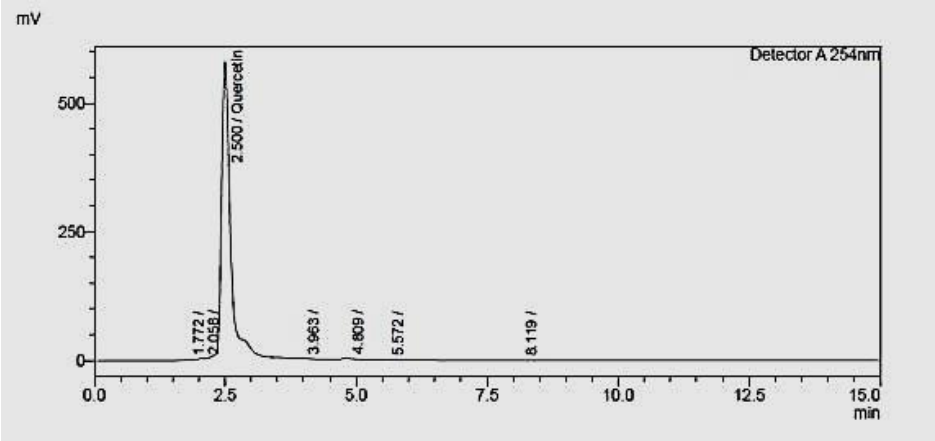


Figure 6. HPLC chromatogram of standard quercetin.

Table 1 Total polyphenol content of lime samples (Mahyuni., 2016).

No	Sample	Content-soluble polyphenol (mg/g gross weight)	Content of Quercetin (µg/g dry weight)
1	Leaf	4,675	92,71
2	Rind	6,954	112,47
3	**Fruit core**	**2,243**	**89,49**

Table 2 Percentage of organoleptic properties selected by respondents.

Organoleptic properties	**Choices**	**Respondent**	Percentage
Scent	Lime	3	30%
	Menthol	1	10%
	Bethel leaf	0	
	Rancid	0	
	Another scent	0	
Texture	Gentle	10	100%
	Other textures	0	
Nature of use	Cool, not sticky, not dry	9	90%
	Cold, sticky, not dry	0	
	Not cold, not sticky, not dry	1	10%
	Other traits	0	
Absorption rate	Fast	6	60%
	Less absorbent	4	40%
	Not fast absorbing	0	
Color	Clear	4	40%
	Greenery	5	50%
	Yellow	0	
	Other colors	1	10%

Table 3. Respondents' Level of Preference.

Organoleptic properties	**Like very much**	**Like slightly**	**Neither like or dislike**	**Dislike very much**
Scent	0	5	5	0
Texture	8	2	0	0
Nature of use	3	6	1	0
Absorption rate	2	7	1	0
Color	6	4	0	0

This organoleptic test observation aims to determine the characteristics of hand sanitizer products that are tailored to the public's taste for hand sanitizers traded in the market. This organoleptic test was carried out using the variables of aroma, color, and characteristics of the use of the product on the hands. From the results of observations made by 10 respondents who were involved in this test, the following data were obtained from the results of organoleptic testing, as seen in the Table 2. And the result of the respondents is in Table 3.

Based on the results of the organoleptic test, the overall bio-hand sanitizer based on lime peel and betel leaf is quite attractive to the respondents. At the level of preference of a total of 10 respondents. Where, as many as 8 respondents liked the soft texture of the bio-hand sanitizer product, as many as 7 respondents liked the fast absorption rate when applied to the respondent's hands, as many as 6 respondents liked the nature of the use which had a cold texture, not sticky but 1 respondent did not like the nature of the use and absorption rate of bio-hand

sanitizer products, as many as 5 people like the smell of citrus and menthol in the content of bio-hand sanitizer products, but as many as 5 respondents of them do not like the aroma of bio-hand sanitizer products, and as many as 6 people like clear colors a little green from a bio-hand sanitizer product based on lime peel and betel leaf.

Figure 7. Bio-Hand sanitizer products based on lime peel and betel leaf.

Figure 7 shows the final product of a based bio-hand sanitizer lime peel and betel leaf. This bio-hand sanitizer product has almost the same characteristics as commercial hand-sanitizer products. The result of this bio-hand sanitizer product produces a clear, slightly greenish color, is cool, soft textured, and has a distinctive citrus and menthol aroma. This causes bio-hand sanitizer products to gain advantages that can increase the attractiveness of the product when traded in the market. The use of bio-hand sanitizer products when applied to the skin of the hands does not cause the skin to become dry and sticky.

CONCLUSIONS

Based on the research that has been done, the following conclusions can be drawn: Betel leaf and lime as natural hand sanitizers contain essential oils and several flavonoids such as quercetin which is a type of flavanol that is capable of being a natural antiseptic and is effective in inhibiting the spread of pathogens and killing pathogens. This is also evidenced by the results of HPLC analysis that orange peel and betel leaf contain quercetin as an antiseptic compound. From the research results, the bacteria that is proven to be able to be killed and inhibited its spread is *Escherichia coli.* In comparison to its effectiveness, if the bio-hand sanitizer product is compared with 70% alcohol commercial hand sanitizer, bacteria will still grow a little in the bio-hand sanitizer, whereas in the commercial hand sanitizer they have not grown. The characteristics of bio-hand sanitizer are almost the same as commercial hand sanitizer products by producing a clear, slightly greenish color, cool, soft texture, and distinctive citrus and menthol aroma, thus gaining advantages that can increase the attractiveness of the product when traded in the market. Bio-hand sanitizer 40% is proven to be capable and effective as an antiseptic against levels of bio-hand sanitizer with 70% ethanol formulation because it produces a hand sanitizer product that is less alcohol than other hand sanitizer products. In a sense, bio-hand sanitizer products based on lime peel and betel leaf are successful in helping to become antiseptic and hand sanitizers from external germs and bacteria due to the assistance of other natural ingredients that can act as antiseptics so that the use of alcohol in hand sanitizer products can be minimized.

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