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Research Article

Synthesis and Characterization of Ethanolic Extract of Red Betel Leaf as an Antiseptic Gel

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ABSTRACT

Hand hygiene is one way to maintain health. There are several ways to clean hands, namely by washing hands and applying a hand sanitizer. The use of hand sanitizer is increasing due to its practical nature. Utilizing natural materials for preparing hand sanitizer widely available around us will be beneficial, one of which is red betel leaf which some people of Indonesia empirically use for antiseptic. This study aims to develop antiseptic gel preparations with ethanolic extracts from red betel leaves. The extraction was done using maceration with 70% ethanol solvent. The formula for choosing a carbomer as a gelling agent with the red betel leaf extract concentration was 0%, 2.5%, 5%, 10%, and 15%. The assessments of the gel were physical and antiseptic evaluations. The physical evaluations included an organoleptic, pH, viscosity, adhesion, and dispersion tests. The antiseptic activity was determined by a replica method. The physical evaluation results of red betel gel revealed that the higher the red betel leaf extract levels are, the darker the green color will be, and the lower the pH and viscosity will be. Furthermore, the antiseptic activity showed that red betel extract gel effectively reduced the number of bacterial colonies.

INTRODUCTION

Health is an important aspect that can affect the quality of life of each individual (Karimi and Brazier, 2016). One effective way to maintain a healthy body is to maintain cleanliness, one of which is hand hygiene (Kumar *et al*., 2020). Hands are a medium for transmitting various diseases. It is caused by viruses, bacteria and fungi that stick to the hands when someone is doing activities (Darmayani *et al*., 2023; Filipe et al., 2021). However, awareness of the importance of washing hands in Indonesian society is lacking. The root of the problem is very simple, namely being lazy or not having time to wash the hands (Yanti *et al*., 2020).

The recommendation to maintain hand hygiene is hand washing with soap, running water, or an alcohol-based hand sanitizer called an antiseptic. Cleaning hands with antiseptics has been known since the early 19th century (Suhono *et al*., 2021). The use of hand antiseptic in gel preparations has become a lifestyle in Indonesian society, especially among the middle to upper classes (Yanti and Futri, 2022). Some gels in hand sanitizer preparations widely distributed in the market usually contain alcohol. The procedure to use it is straightforward: dripping on the palm and then flattening it to all parts of the palm. The community more widely uses gel preparations since they are easy to use and have good aesthetic value. They are transparent, easily spread when applied to the skin, give a cold sensation, and do not cause scars (Anisah, 2014).

Alcohol, the main ingredient in hand sanitizer, is often avoided by some people; thus,

it is necessary to find a replacement for it (Golin *et al*., 2020). Abundant natural materials around us can be used as alternative materials. Indonesia has abundant biodiversity and is called a mega-biodiversity country (Choy, 2015). One of the biodiversity that can be developed as an antiseptic is red betel leaf (*Piper crocatum* Ruiz. & Pav.), which reportedly contains flavonoids (Saputra *et al*., 2016). This compound may be effective as an alcohol substitute for making hand-sanitizing gel. This paper aims to identify the extraction of the active ingredient of red betel, antiseptic gel formulation, physical gel test, and antiseptic gel test. This research is one of the steps in making a hand sanitizer based on the efficacy of red betel extract as an antibacterial, as stated by previous researchers (Chairunisa *et al*., 2022; Hartini *et al*., 2018).

MATERIALS AND METHODS

Materials

The red betel leaves were obtained from the Secang area, Magelang, Central Java. The 70% ethanol, carbomer, glycerin, triethanolamine (TEA), and distilled water were purchased from Bratachem®. Tryptic soy agar (TSA), acetic acid, rutin, and n-butanol were obtained from Merck®.

Extraction

Red betel leaves were sorted and washed to remove the dirt using clean water and then dried. The next process was refining (powdering) and screening. These two processes aimed to obtain a homogeneous simplicia powder and facilitated the withdrawal of its active compound that could be used as an antibacterial, such as a flavonoid (Hartini *et al*., 2018). The red betel extracts were made using the maceration method. The solvent used was 70% ethanol, with a ratio of 1:10 between the red betel powder and the solvent used (Sari and Isadiartuti, 2006). Furthermore, the liquid was evaporated using rotary evaporation until a thick extract was formed. The ethanol-free test was conducted qualitatively by reacting the ethanolic extract of red betel leaves with two drops of concentrated sulfuric acid (H_2SO_4) and 1 mL of potassium dichromate ($K_2CR_2O_7$). Changes in color from orange to bluish-green indicated positive containing ethanol (Robinson, 1995). The flavonoid content test was performed using Thin Layer Chromatography (TLC). As a comparison, it used rutin as the standard. The mobile phase for TLC was n-butanol: acetic acid: water (BAA) with a 4: 1: 5 v/v ratio. The TLC stationary phase used was cellulose. Detection was done using visible light, ultraviolet (UV) light 254 nm, and UV light 366 nm (Harborne, 1987).

Formulation and Evaluation

The antiseptic gel's formulation of ethanolic extracts of red betel leaves can be seen in Table 1. The initial step in manufacturing red betel extract antiseptic gels was developing a carbomer base. Carbomers are developed using hot water. Betel leaf extract is dissolved with glycerol until completely dissolved, and then the extract is mixed into the carbomer base that has been developed. The dissolved methyl and propyl parabens were added in sufficient alcohol and stirred until homogeneous. The final step was adding water to the desired volume, then adding TEA little by little while stirring slowly until homogeneous. The evaluation of the resulting gel included a physical test and an antiseptic test. The antibacterial activity test was conducted using the replica method. The hand sanitizer gel of red betel extract and the Carex hand sanitizer (gel's bases and positive control) were applied or dripped on the cleaned palms.

Table 1. Antiseptic gel formula of red betel leaf extract

Ingredients	F1	F2	F3	F4	F5
Extract	0%	2.5%	5%	10%	15%
Carbomer	1%	1%	1%	1%	1%
Glycerin	3 %	3 %	3 %	3 %	3 %
TEA	1.5%	1.5%	1,5%	1.5%	1.5%
Methyl Paraben	0.18%	0.18%	0.18%	0.18%	0.18%
Propyl Paraben	0.02%	0.02%	0.02%	0.02%	0.02%
Distilled water add	100 mL	100 mL	100 mL	100 mL	100 mL
Ethanol 70 %	2 mL	2 mL	2 mL	2 mL	2 mL

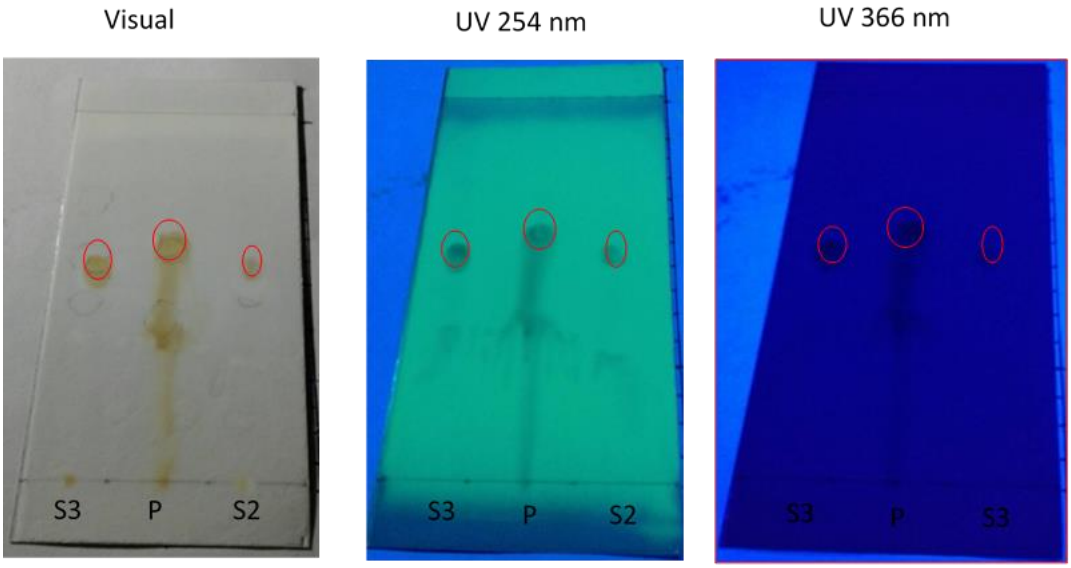


Figure 1. TLC results for flavonoid compounds. (S3) red betel extract with three-time spotting. (P) Rutin. (S2) red betel extract with two-time spotting.

After 1-2 minutes, the palms were swabbed using a wet sterile cotton bud and then swabbed in the growth media (TSA), continued by incubating for 24 hours at 37°C. The antibacterial activity was evaluated by counting the number of colonies in the growth medium (Ramayani *et al*., 2021).

RESULTS AND DISCUSSION

Extraction

The plant used in this study was red betel (*Piper crocatum* Ruiz. & Pav.) obtained from the Secang area, Magelang, Central Java. Determination of the plant was done at the Laboratory of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada (UGM). Plant determination aimed to establish authentication related to the plants' characteristics, such as macroscopic and microscopic morphological features compared to literature. The determination of number BF/47/Ident/Det/II/ 2015 showed that the simplicia used was red betel (*Piper crocatum* Ruiz. & Pav.). During the extraction processes, the result of the yield was 12.5%. The yield produced in this study was similar to that of red betel extract conducted in a previous study of 11.92% (Kanifah *et al*., 2015).

An ethanol-free test aims to prove there was no ethanol in the red betel leaf extract. Thus, the output of antiseptic power was due to the influence of the concentration of red betel extract, not from the ethanol solvent compound of red betel extract. Based on the test results, it was found that there was no discoloration from orange or red to bluish-green, indicating that the extract of red betel leaves was qualitatively free from ethanol. The reactions between potassium dichromate, sulfuric acid and ethanol-in this test are shown in Equation 1.

$$K_2Cr_2O_7 + 4H_2SO_4 + 3CH_3CH_2OH \rightarrow Cr_2(SO_4) + K_2SO_4 + 7H_20 + 3CH_3CHO \quad (1)$$

The test for identifying flavonoid content in red betel leaf extract was conducted using the TLC method, followed by a densitometry test. The solution spotted in the stationary phase resulted from the dilution of the extract and the comparison solution. The solution compared was rutin (Anwar and Triyasmono, 2016). Elution results were then observed in visible light, UV 254 nm, and UV light 366 nm (Rahman, 2007). Figure 1 shows the result of the identification of flavonoid compounds by the TLC method.

According to previous research, flavonoid compounds would produce yellow spots on the results of the TLC elucidation as observed in visible light. They would produce yellow spots that fluoridated when detected using UV light 254 (Sudarmanto and Suhartati, 2016). The results of this study align with the theory that the sample produced fluorescing yellow spots when detected using UV light 254. The Rf obtained was 0.72 in rutin solutions and 0.63 in extract solutions. The Rf value was included in the range of flavonoid compounds' value, which was between 0.2 - 0.75. When the Rf range is between 0.6 - 0.75, it is included in the range of quercetin compounds (Mursyidi, 1990). The difference in

the Rf value between the extract and rutin solutions in this process may be because the rutin is more polar than quercetin compounds from red betel extract. It may have occurred that the number of OH groups in rutin compounds was more than those found in quercetin compounds of red betel extract.

Formulation and Evaluation

Optimization of bases for gel preparation in this study aims to determine the concentration of carbomer that will be used. The highest inhibition of red betel ethanolic extract on positive and negative gram bacteria growth was at a concentration of 6.25% b/v. Thus, based on previous research, this study used the red betel ethanolic extract 6.25% b/v to optimize the carbomer bases gel preparation (Soleha *et al*., 2015). A decent analysis of the stock base refers to the formula's viscosity value, which approaches the viscosity value of the positive control. The results of the optimization can be seen in Table 2.

Based on the Handbook of Pharmaceutical Excipients, the use of carbomer as a gelling agent was at a concentration of 0.5-2% (Rowe *et al*., 2009). Thus, the optimization was done using three concentration variations: carbomer concentration of 0.5%, 1%, and 1.5% (Ida and Noer, 2012). This study used it as a reference for conducting viscosity measurements on each preparation. The measurement results showed that the viscosity of gel preparations that was close to the viscosity of control preparations was gel preparations with a base concentration of 1% with a viscosity of 6.92 poise. The results were then used as a reference to design the antiseptic formulation of red betel extract gel.

The antiseptic formula of red betel leaf extract was designed using a varied concentration of red betel extract ranging from 0% to 15%. The concentration variation was selected to determine whether there were differences in antiseptic activity between the concentration of red betel extract before and after it became a gel preparation. Based on the optimization of the red betel extract antiseptic gel, the carbomer concentration to be used was 1%. The formulation design for this study referred to a previous study (Sari and Isadiartuti, 2006). Based on the formulation, some modifications were made to the ingredients. The modifications included adding preservatives and modifying the concentration of each ingredient (based on the concentration of the ingredients used in the optimization of the red betel extract antiseptic gel). The amount of material used for the formulation can be seen in Table 3.

Table 2. Optimization of antiseptic gel preparation of red betel extract

Bases	Viscosity (Poise)
Carbomer 0.5%	4.21
Carbomer 1%	6.92
Carbomer 1.5%	39.50
Positive Control (Gel "Carex")	10.30

Table 3. Calculation of amount of material for gel preparation

Ingredients	F1	F2	F3	F4	F5
Extract	0 g	2.5 g	5 g	10 g	15 g
Carbomer	1 g	1 g	1 g	1 g	1 g
Glycerin	3 mL	3 mL	3 mL	3 mL	3 mL
TEA	1.5 mL	1.5 mL	1.5 mL	1.5 mL	1.5 mL
Methyl Paraben	0.18 g	0.18 g	0.18 g	0.18 g	0.18 g
Propyl Paraben	0.02 g	0.02 g	0.02 g	0.02 g	0.02 g
Ethanol 70%	2 mL	2 mL	2 mL	2 mL	2 mL
Distilled water	91.74 mL	88.97 mL	86.47 mL	81.47 mL	76.47 mL

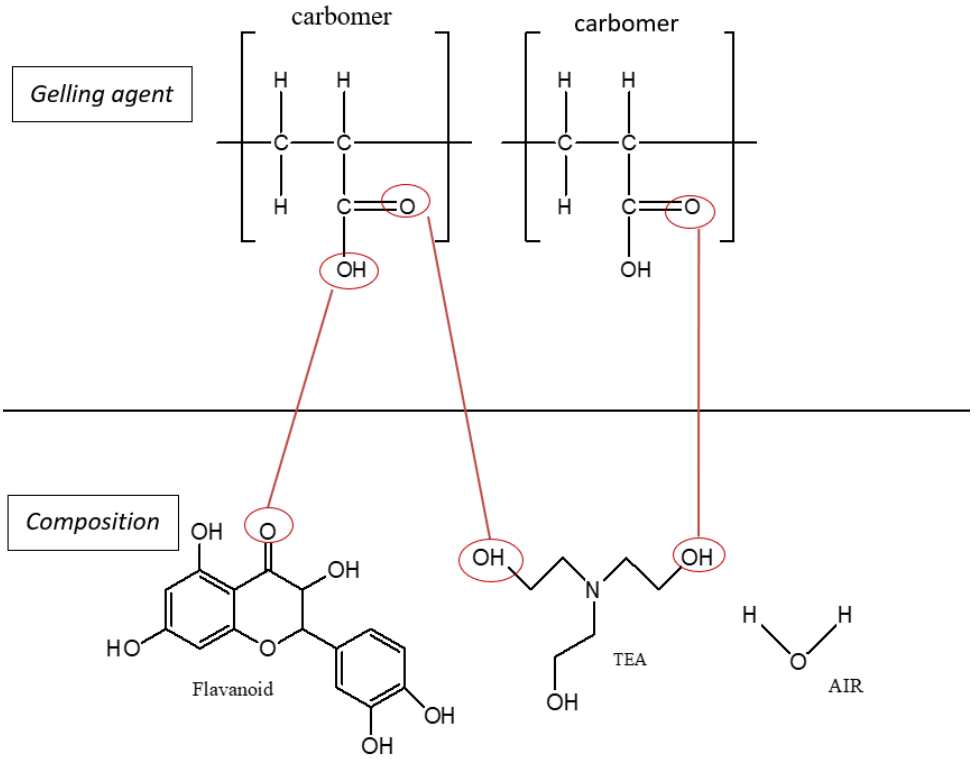


Figure 2. Proposed illustration of interactions between carbomer, flavonoids, TEA and water (Lovett *et al*., 2015).

The formulation calculated the number of ingredients or materials in a liquid form and considered the density of each material. It was to calculate the amount of distilled water that would be added to the formulation. The density of distilled water included ethanol 70%, TEA, and glycerin were 1 g/mL, 0.886 g/mL, 1.124 g/mL, and 1.29 g/mL, respectively. This study prepared the red betel extracts antiseptic gel as a one-phase system gel. The stability of gel preparations could be observed visually in a gel form. A good gel preparation is a preparation that can maintain a smooth and regular distribution of organic macromolecules as active substances over a long period.

In developing a base, when the carbomer is mixed with water in an acidic solution, it forms a gel mass with a strong affinity between the active substance and the water-containing base. Affinity is the tendency of a compound to form a bond with another compound. Thus, the active water-soluble substance in the preparation is difficult to escape and has difficulty penetrating bacteria. TEA was added as an adjusting pH or ionizing agent that would ionize and cause the water-soluble active substance to enter and be trapped in a matrix and easily rereleased; thus, it could easily penetrate. The characteristics of the carbomer were hydrocolloid or hydrophilic, indicating that if it is dispersed in water, it will expand.

The following process was molecular hydration through the formation of hydrogen bonds. Carbomers have chemical compounds whose ends of the chain have acidic RCOOH groups; some carboxyl groups in the carbomer molecular structure will form unionized coils. If adding a base increases the pH of the carbomer dispersion, the carboxyl group will progressively ionize. The ionization process of this carboxyl group will result in repulsion between ionized groups and cause hydrogen bonds in the carboxyl group so that the viscosity increases (Lovett *et al*., 2015). The illustration of the interaction between carbomer and material (active substances, such as flavonoids, water, and TEA) can be seen in Figure 2.

The interaction of hydrogen bond formation between carbomer, TEA, and components of red betel extract occurred due to the gelling agent's hydroxyl group (-OH) and carbonyl group (C=O). The more hydrogen bonds that are formed, the higher the viscosity will increase.

The stability tests of the red betel extracts of antiseptic gel were conducted by an

organoleptic, homogeneity, pH change, viscosity, stickiness, and dispersion tests. The results of the stability tests can be seen in Table 4.

Organoleptic and Homogeneity Tests

An organoleptic test is often conducted as quality control of a preparation. This test is usually done to visually determine the presence or absence of changes from preparations stored within a specific time. In this study, the organoleptic test results revealed a red betel leaf extract gel formula that was light green at a concentration of 2.5% and dark green at formulations of 5%, 10%, and 15%, with a typical red betel odor. The color of the gel for all the formulas can be seen in Figure 3.

The next test was a homogeneity test of gel preparations, which is an essential factor in determining the quality of the preparation. The test aims to identify the uniformity of the gel preparation particles to produce the maximum effect. The results for the homogeneity test showed that the antiseptic gel betel extract had a good homogeneity, characterized by observations in which all the particles in the gel preparation were evenly dispersed on the slide, and there was no clumping of particles when observed under a microscope. Furthermore, based on the evaluation of its color, odor, and homogeneity, the preparation remained stable until the 7th week of testing.

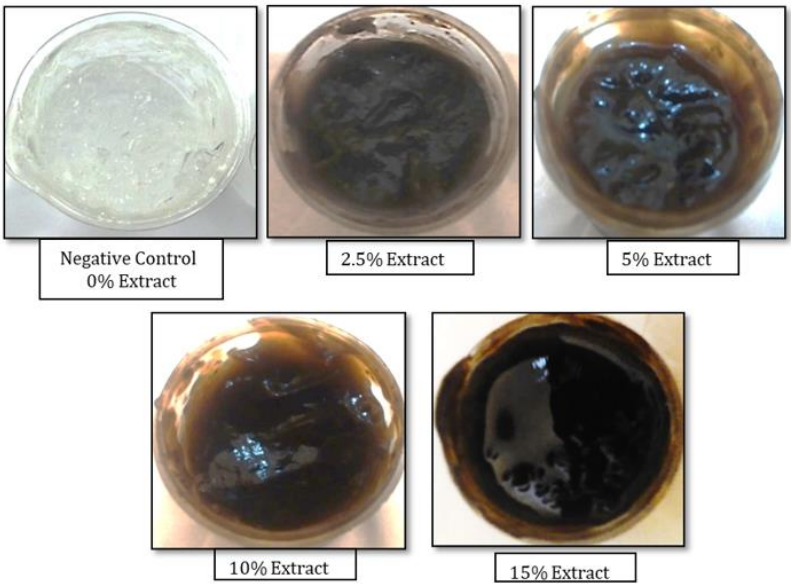


Figure 3. Color of red betel extract at various concentrations

Table 4. Data of gel's characteristic red betel leaf extract test for 7 weeks

Characteristics	Formula					
	Positive Control	F1	F2	F3	F4	F5
Color	No-color	Clear	Dark green	Dark green	Dark green	Dark green
Smell	Alcohol	Typical Base	Typical Red Betel	Typical Red Betel	Typical Red Betel	Typical Red Betel
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
pH	5.71± 0.03	6.68±0.45	5.88±0.24	5.69±0.19	5.28±0.13	5.09±0.05
Viscosity *	-	2468.89±248.10	1545.67±286.17	592.22±128.03	324.11± 115.75	99.11±31.46
Adhesion **	-	0.81±0.02	0.79±0.02	0.46±0.06	0.31±0.05	0.20±0.04
Scattering Power	-	1.82	2.12	2.20	2.47	2.72

Viscosity Test

Viscosity is a measure of viscosity that indicates the amount of friction in the fluid. The greater the viscosity of a fluid is, the harder it will be for an object to move in a fluid. In this case, the thicker the gel preparation is, the greater the strength that will be needed for the gel preparation to flow at a certain speed (Martin *et al*., 2011). Increasing the viscosity of the gel will reduce its spreading power, which will certainly reduce the comfort in application to the whole hand as a hand sanitizer (Yusuf *et al.*, 2017). A proper viscosity value is considered to be in the range of 2000-4000 cps (Garg *et al*., 2002). In addition, with the preparation's higher viscosity, the dispersed phase's separation rate was lower; thus, the gel preparation was more stable (Suryani *et al*., 2000). Figure 4 shows that the higher the concentration of red betel extract is, the smaller the viscosity of the gel preparation will be.

One factor that influences the viscosity of gel preparation is pH. In this case, carbomer has a stable level of viscosity at pH 6-11(Warnis *et al*., 2023), in which the consistency (thickness) is produced due to the addition of TEA to the preparation so that the carboxyl group owned by the carbomer will turn into COO- (Ismail *et al*., 2021). In addition, there will be a resisting electrostatic repulsion between the ionized group, causing the hydrogen bond to become stronger; thus, it causes the carbomer to expand, becoming more rigid and stable (Lovett *et al*., 2015).

In this study, the viscosity test of the preparation showed that the higher the red betel leaf extract content is, the more the viscosity of the preparations will decrease. It occurred due to several factors: the pH of the carbomer, the pH of the extract, and the amount of TEA. When the carbomer pH has been developed, ranging from 2-4, to produce a good gel preparation, sufficient TEA is needed and functions as a thickener, purifier, and pH neutralizer (pH 7). However, in the red betel gel preparation, the extract's pH was acidic, which was 4.06. Therefore, an additional amount of TEA was needed to make the gel preparation. However, in this formulation, an equal amount of TEA was used at each increase in the concentration of the extract, which was prepared with high concentrations of acid. It also resulted in the number of decreased ionized carboxylic groups, and the repulsion of the carboxyl group caused the development of the carbomer structure to decrease. As a result, it caused a decrease in the viscosity of the gel preparation with an increasing amount of extract. Red betel extract gel preparations that fell within the ideal viscosity range were formula 1 (F1) or formula without red betel extract (0%). As for formulas 2, 3, 4, and 5, although they did not meet the ideal viscosity value, they still maintained stability until the 7th week.

pH Test

The function of measuring the pH of gel preparations is to determine the preparation's stability and whether the preparation is safe or not irritating when used on human skin. Carbomer has a stable viscosity level at pH 6-11 (Warnis *et al*., 2023), and the skin's pH ranges from 4.5 to 6.5 (Ali and Yosipovitch, 2013). Meanwhile, flavonoid stability was reported in acidic conditions since the flavonoid was a weak acid (Yao *et al*., 2014). Based on the study results, the pH of the preparations included in the pH range of carbomer stability was the pH of formula one (F1).

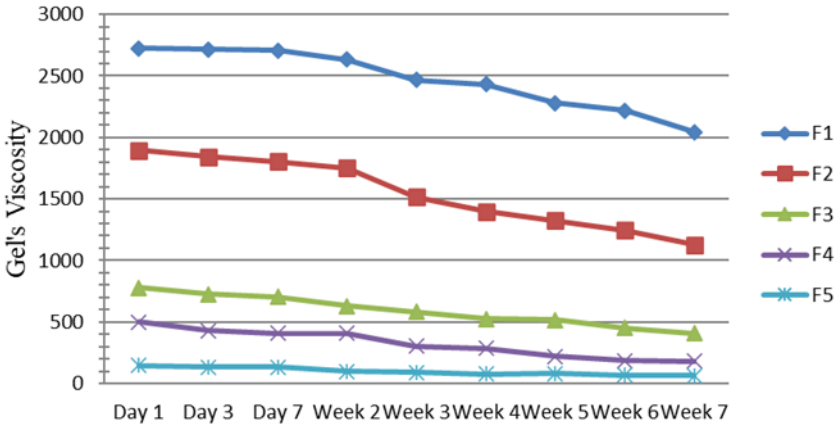


Figure 4. Viscosity test of antiseptic gel betel extract preparation in red betel

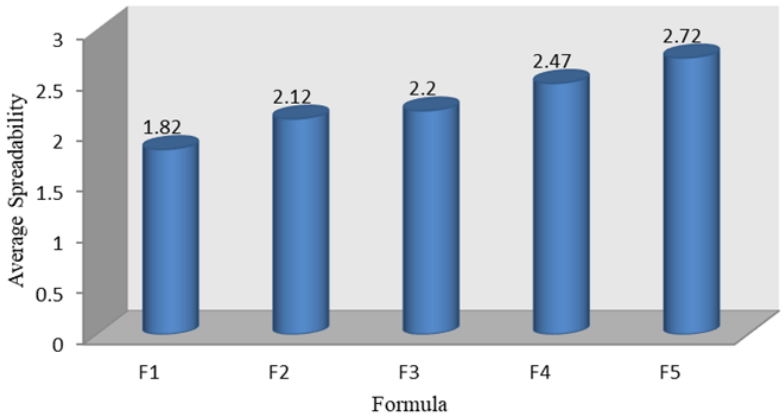


Figure 5. Average spreadability of red betel extract antiseptic gel

In contrast, the pH within the skin pH range was positive control gel, formula 2 (F2), formula 3 (F3), formula 4 (F4), and formula 5 (F5). However, the difference between the pH of the gel and the skin's pH will not irritate the skin or damage the skin because the skin has a high buffer capacity. Thus, a temporary pH change will occur if the skin is exposed to an acidic or basic material or solution. However, skin pH will return quickly to normal, indicating the skin has a high buffer capacity (Levin *et al*., 2001).

Spreadability Test

The purpose of testing the spreadability of the gel preparation in this study was to determine how the gel spreads on the skin so that the spread of active substances from the gel preparation could be identified. A spreadability test is one of the requirements of semisolid preparation. Supposing the semisolid preparation has a high spreadability, then in that case, it will cover a wide area on the skin so that the active substances contained in the semisolid preparation will be spread evenly. A proper gel spreadability is between 5 and 7 cm (Sugihartini and Wiradhika, 2017). Spreadability was caused by the composition of the red betel extract in the preparation. According to the spreadability test result, the higher the extracted content is, the bigger the spreadability will be. In this study, the results of the spreadability test showed that the preparations with the greatest spreadability was the 15% red betel extract gel with an average of 2.72 cm. The spreadability and average spreadability results at the first observation can be seen in Figure 5.

Adhesion Test

The adhesion test aims to determine the gel's ability to adhere to the skin. Good adhesion can coat the skin as a whole and does not complete the physiological function of the skin, and clog pores (Hwang *et al*., 2018). Good gels have a high adhesion (Sun *et al*., 2021). The adhesion of preparation is affected by the viscosity of preparation, in which the higher the viscosity is, the higher the adherence will be and the opposite. Sources of subscription are needed to identify the duration of active substances' effect from a preparation (Nurlaela *et al*., 2012).

In this study, the adhesion from red betel extract testing decreased. The resulting drop pattern was F1> F2> F3> F4> F5. The influencing factors were the pH and the viscosity of the preparation. Increasing the viscosity of the preparations made the preparations thicker, which caused an increase in the adhesion of the gel and vice versa. If the viscosity decreased, so did the adhesion. Regarding the preparation of red betel extract gel, the result of the adhesion value was not included in the ideal value range of adhesion due to the excessively liquid dosage form.

Antiseptic Test of Red Betel Extract Gel

The replica test was used to identify the effectiveness of the red betel extract's antiseptic (Iskandar *et al*., 2021). The replica test was done by dripping and leveling the gel preparation on the palm and then swabbing a sample from the palm to the TSA media. The media were incubated for 24 hours at 37^0C, and the colonies growing on the media were counted (Ramayani *et al.*, 2021).

Table 5. Results of antiseptic activity test of red betel extract gel

Preparation	Average bacteria growth		Average bacteria reduction (%)	Significance Dependence T-test	Normality test
	Before	After			
Control (+)	582	5	99.14 %	0.000	0.957
Control (-) *	583	571	1.38 %	0.781	0.704
2.5 %	732	711	2.89 %	0.118	0.487
5 %	673	490	27.19%	0.000	0.657
10 %	997	318	68.10%	0.000	0.313
15 %	598	86	85.62 %	0.000	0.024

* Control (-) = red betel extract gel 0%
* Control (+) = antiseptic hand sanitizer gel "Carex".

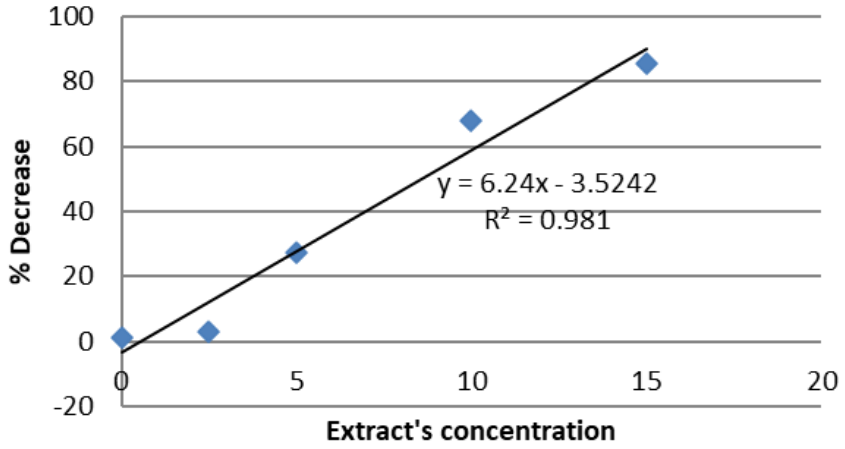


Figure 6. Antiseptic test (effect of increased extract concentration vs. % number of decreased bacterial colonies).

The antiseptic test in this study aims to determine how much the decrease in the number of germ colonies on the palm before and after being given the antiseptic of red betel extract gel and the commercialized antiseptic gel as a positive control. Based on Table 5, regarding the results of the antiseptic tests of red betel extract gel, there was a decrease in the growth of the number of colonies after using red betel extract gel preparations. Significant reduction in the number of colonies started from the 5% level, 27.19%, the 10% level, 68.10% and the 15% level, 85.62%. Meanwhile, commercial antiseptic gel preparations in the market showed that the number of growing colonies reduced to 99.14%.

Regarding the results, the red betel leaf extract gel with 15% content had antiseptic power, although the effectiveness of the red betel extract gel still lacks compared to the comparative preparation. Figure 6 shows the effect of increasing the extract concentration on the percentage decrease in the number of bacterial colonies. The relationship between the concentration of red betel extract with a decrease in the number of colonies gives Equation 2.

$$Y = 6.24X - 3.5242 \quad (2)$$

The equation explains that the higher the extract concentration is, the higher the decrease in the number of bacterial colonies that will be produced. The value of R produced was 0.981, indicating that it was included in the range of R-value = 0.80 – 1.000. Therefore, it can be concluded that the relationship between the concentration of red betel extract (X) and the percentage decrease in the number of bacterial colonies (Y) is powerful.

For statistical analysis, a paired sample t-test was then conducted, which aimed to identify the significance of a decrease in the number of colonies before and after using the antiseptic gel of red betel leaf extract. Data on the significant value of the number of colonies reduction before and after using the red betel extract antiseptic gel showed that there was a very significant

decrease in the number of colonies before and after using the antiseptic gel on the positive control gel, formula three gel, formula four gel and formula five (Table 5). These results are similar to another research which observed the fire mangrove leaves extract as the antiseptic gel. The increase in extract concentration decreased the number of bacteria colonies (Titaley, 2014).

On the other hand, gel formula one and gel formula 2 showed results that did not significantly decrease the number of colonies. After the paired-sample t-test, the next test conducted was the sample normality test. Based on the normality test data, formulation 5 (F5), which had a significance value <0.05, indicated that it was not expected. Thus, to compare positive control gels, F1, F2, F3, F4, and F5, a nonparametric test was conducted, namely the Kruskal-Wallis test, followed by the Mann-Whitney test. The Kruskal-Wallis test was conducted first to determine whether there were significant differences between preparations. Meanwhile, if the value of the Kruskal-Wallis test showed a significant difference, it would be continued with the Mann-Whitney test to identify the significance value between positive control gels, F1, F2, F3, F4, and F5. Furthermore, the next test was the Kruskal-Wallis test. Based on the test data, there was a significant difference between all preparations (significance value <0.05). Mann Whitney test was then done to identify the difference between positive control gels, F1, F2, F3, F4, and F5. Based on the Mann-Whitney test results, it can be concluded that there was a significant difference between positive control gels against F1, F2, F3, F4, and F5.

The results of the significance of the Mann-Whitney test indicated that the insignificant difference only occurred between formula 1 (negative control or 0%) and formula 2 (2.5%). The last statistical analyses conducted were correlation and regression tests between F1, F2, F3, F4, and F5 dosage formulations. The correlation test aimed to determine whether there was a linkage to the sample. In this case, the relationship between the concentration increase of the extract and the number of bacterial colonies decreased. The correlation test was performed using the Pearson correlation test as the selection of correlative hypotheses was based on the data type, and the two variables tested were numerical.

Based on the correlation data between F1, F2, F3, F4, and F5, it was found that there was a significant correlation between the two variables (formula and reduction). The result of the correlation strength level indicated very strong (0.80 - 1.00) with a value of 0.982, while the direction of the resulting correlation was positive. It implied that the greater the red betel leaf extract concentration is, the more significant the decrease in the number of colonies will be produced.

Moreover, a regression test was conducted to examine the effect of increasing concentrations of red betel extract on the decrease in the number of bacterial colonies. The regression test conducted was a simple regression test where two variables were tested: one as the dependent variable, namely, the decrease in the number of colonies, and one independent variable, namely, the formula. Based on the test, the significance value obtained was 0.003, indicating an influence between an increase in the concentration of red betel extract in the formula and the decrease in the number of bacterial colonies. After examining the effect between the concentration of red betel extract increased in the formula and the decrease in the number of bacterial colonies, the effect showed 6.240. Therefore, the regression equation is expressed in equation 3.

$$Y= 6.240X -3.524 + e \quad (3)$$

Equation 3 explains that if there is an increase of 1 unit in the concentration of red betel extract in the formula, the decrease in the number of germs will increase by 6.240 while the resulting R square value is 0.964. It indicates that an increase influences the 96.4% decrease in the number of bacterial colonies in the concentration of red betel extract in the formula. Therefore, equation 3 can be used to predict levels to decrease the number of bacterial colonies by 100% (Y = 100%) at a concentration of 16.69 or 17%.

CONCLUSIONS

Based on the results of this study, it can be concluded that the gel's appearance was green and met the standard gel requirement: viscosity, pH, spreadability, and additional strength. Fractionation of the extract may need to be conducted to increase the appearance. The red betel extract gel effectively reduced the number of bacterial colonies up to 85.62% at a concentration of 15%. Meanwhile, for positive control, the number of bacterial colonies decreased by 99.14%. The regression analysis showed 100% bacteria colony removal might occur when the red betel leaf extract concentration was 17%.

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CONFLICT OF INTEREST

All the authors declare that there is no conflict of interest.

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