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# Optimization of hand sanitizer gels containing soursop (*Annona muricata* L.) leaf extract using simplex lattice design method

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**ABSTRACT**: Soursop (*Annona muricata* L.) is a plant whose leaf extract contains alkaloids, flavonoids, saponins, and tannins and can be antibacterial. Based on the compounds it contains, soursop leaf extract can be an active substance in hand sanitizer gel preparations. This study aims to determine the characteristics of the resulting hand sanitizer gel and the optimum concentration of the base combination that can produce a qualified hand sanitizer gel. This study used a Simplex Lattice Design experimental method with two factors (carbomer 940 and HPMC). Three hand sanitizer gels were prepared, with a ratio of carbomer 940: HPMC for F1 (1%:0%), F2 (0.5%:0.5%), and F3 (0%:1%). Tests conducted on hand sanitizer gel preparations included organoleptic, homogeneity, spreadability, adhesion, pH, and viscosity. The results of physical properties tests (spreadability, adhesion, and viscosity) were analyzed using Design Expert 11. The results showed that carbomer 940 and HPMC could increase viscosity and adhesion but could decrease spreadability. Ethanol extract of soursop leaves (*Annona muricata* L.) could be formulated and optimized into hand sanitizer gels. The optimum formula contained carbomer 940 0% and HPMC 1%, obtained in Formula 3 with a desirability value of 0.986.

**KEYWORDS**: Hand sanitizer gel; optimization; soursop leaves (*Annona muricata* L). leaves; simplex lattice design.

## INTRODUCTION

As a tropical country, Indonesia has abundant natural wealth. One of the natural wealth owned is herbal plants. Indonesian people generally use herbal plants to prevent diseases that are used based on heredity. One of the herbal plants widely used by the community is the soursop plant [1].

Soursop (*Annona muricata* L.) is a tropical plant with a distinctive taste and smell. The parts of the soursop plant used as medicine are leaves, flowers, fruit, seeds, roots, and barks [2]. Soursop leaves possess antibacterial and antifungal properties and are effective against various worms and parasites. Soursop leaf extract has shown antibacterial efficacy against hand-transmitted pathogens like E. coli and S. aureus in various studies. Specific data includes inhibition zone diameters ranging from 6.59 to 12.3 mm for E. coli at 0.5% and 1% concentrations. Minimum inhibitory concentrations (MIC) have been reported as 6.25% for S. aureus [2]. Soursop leaves contain active compounds of alkaloids, flavonoids, saponins, and tannins that have antibacterial activity against *Staphylococcus aureus* [3].

*Staphylococcus aureus* is a bacteria that can attack human health because it often contaminates the hands. Moreover, bacteria can be harmful because there are no hand-washing habits [4]. A practical method to maintain hand hygiene is by using a hand sanitizer.

Hand sanitizer is a preparation used to reduce or inhibit the growth of microorganisms. It is highly effective and practical because it does not require water for rinsing and where soap and water are absent. Hand sanitizer preparations available on the market contain the active ingredient alcohol. The choice of alcohol in the formulation is because alcohol has a bactericidal activity that works against several types of bacteria but not against viruses and fungi. Alcohol can also provide a fresh feeling in the hands and make the hand sanitizer gel dry faster when used [5],[6].

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Based on the previous research [2], soursop leaf extract was made into hand sanitizer gel preparations using carbomer 940 as the base. The study results showed good physical characteristics such as organoleptic, spreadability, pH, and homogeneity. The research mentioned that ethanol extract from soursop leaves can inhibit the growth of *Staphylococcus aureus* bacteria at an extract concentration of 12% with an inhibitory diameter of 22 mm and has the very potent category. Gelling agents commonly used are hydroxypropyl methylcellulose (HPMC) and carbomer 940. HPMC is a gelling agent used for cosmetic and medicinal gel production. HPMC is stable at pH 3 to 11, safe, and does not cause skin irritation. Carbomer 940 is a stable, hygroscopic synthetic polymer used for cream, gel, lotion, and ointment preparations. Carbomer 940 is non-toxic, non-hazardous, and does not cause hypersensitivity in topical use [7]. Also, carbomer does not irritate and is suitable for gel preparations containing alcohol and water [5].

The combination of carbomer 940 and HPMC was optimized using the Simplex Lattice Design method. This method aims to avoid trial and error in formulation. Then, to find an optimum formulation. The method can help reduce the energy, cost, and time needed [8].

## ▪ MATERIALS AND METHODS

### Materials

Soursop leaves (Balitro, Indonesia), purified water (Resia Niaga, Indonesia), carbomer 940 (Sumitomo, Japan), hydroxy propyl methyl cellulose (Kehao, China), triethanolamine (Mallinckrodt, USA), 96% ethanol (Mallinckrodt, USA), methyl paraben (SC Johnson, USA), EDTA (Merck, Germany), glycerin (Allied, India), green tea flavor (Samaara, India), and other chemicals are cosmetical or pharmaceutical grades.

### Extraction of soursop leaf samples

Samples of soursop leaves (*Annona muricata* L.) were collected from the Research Institute for Spices and Medicinal Plants (BALITTRO), and determination was at the Plant Conservation Center of the Botanical Gardens - LIPI, Bogor. The leaves picked were washed clean using running water, drained, and cut into small pieces. Then, dried in an oven at 40 °C. The dried leaves were ground by blending until they formed powder. One part of the soursop leaf powder was added to ten parts of the 96% ethanol or 3 kg of the soursop leaf powder was added into 30 L of 96% ethanol solvent for 3 days while stirring several times, then filtered to separate the dregs and macerate. The dregs were re-macerated twice with the same solvent at 3 days each. The macerate was evaporated with a rotary evaporator to obtain a thick extract, weighed, and its yield was calculated [9].

### Loss on drying of soursop leaf extract

The extract was placed in a pre-heated, tared, and closed weighing bottle and then heated in an oven at 105 °C for 30 minutes. Before being weighed, the extract in the bottle is leveled with the help of a stirring rod, put in the oven, opened the cover, and dried at 105 °C until the weight remains constant. After removing it from the oven while still closed, allow it to cool in a desiccator to room temperature. Record the permanent weight, then place it in the oven at 105 °C for 1 hour [10].

### Phytochemical screening of soursop leaf extract

a) Alkaloids: 0.5 g of the extract, 1 mL of 2 N HCl, and 9 mL of purified water were mixed, heated in a water bath for 2 minutes, and cooled. Filtered to produce the filtrate, piped into 2 test tubes with three drops each. Positive alkaloids if two drops of Mayer's reagent were added into tube #1 and resulted in a white or yellow precipitate, or if two drops of Dragendorff's reagent were added into tube #2 and resulted in a brick-red precipitate [11].

b) Flavonoids: 2 g of the extract was added with 6 mL of hot water, then boiled for 5 minutes and filtered. Next, the filtrate was added with 0.05 mg of magnesium powder and seven drops of concentrated HCl, then shaken vigorously. Positive results if colors red, yellow, or orange were visible [12].

c) Saponins: 0.5 g of the extract was added to 10 mL of hot purified water, then cooled and shaken vigorously for 10 seconds. Foam formed that did not disappear for 10 minutes as high as 1 to 10 cm. On the addition of 1 drop of 2 N HCl, the foam did not disappear [11].

d) Tannins: 0.5 g of the extract was boiled for 3 minutes in 10 mL of hot pure water, cooled, and filtered. The filtrate was added with 1-2 drops of 1% $FeCl_3$ to form a blackish-blue or blackish-green solution [13].

**Hand sanitizer gel preparation**

**Table 1.** Optimization of hand sanitizer gel formula from soursop leaf ethanol extract.

| Materials | Formula | | |
|---|---|---|---|
| | F1 | F2 | F3 |
| Soursop leaf extract (g) | 12 | 12 | 12 |
| Carbomer 940 (g) | 1 | 0.5 | 0 |
| HPMC (g) | 0 | 0.5 | 1 |
| TEA (g) | 1 | 1 | 1 |
| Methyl paraben (g) | 0.3 | 0.3 | 0.3 |
| EDTA (g) | 0.01 | 0.01 | 0.01 |
| Purified water (g) | 25 | 25 | 25 |
| Green Tea flavor (drop) | qs | Qs | Qs |
| Glycerin ad | 100 | 100 | 100 |

The first step of the process was preparing the materials and tools, weighing all the materials, and adding EDTA to purified water. In the second step, the gel base consisting of carbomer 940 and HPMC was developed with 25 mL purified water and waited until the base expanded for 30 minutes. Then, stir quickly in a beaker glass until a gel mass forms. In the third step, 0.3 g of methylparaben previously dissolved in glycerin was put into the beaker glass and stirred until homogeneous. The extract added glycerin, put into the beaker glass, and then stirred until evenly mixed. In the next step, TEA was added and stirred until homogeneous. Finally, glycerin addition was up to 100 g and was mixed until the hand sanitizer gel formulation formed [4]. The hand sanitizer gel was stored at 40 ˚C in a climatic chamber for 28 days. The gel evaluations were carried out on days 0, 1, 7, 14, 21, and 28 [14]. Observations on the hand sanitizer gel include:

**Organoleptic test**

The organoleptic test was to observe physical changes by evaluating its color, odor, and shape [15].

**Homogeneity test**

The gel homogeneity test was by applying 0.1 g of the gel on a glass slide, and then the gel appeared homogeneous without any visible coarse grains [9].

**Spreadability test**

A 0.5 g sample of hand sanitizer gel was placed on a transparent glass surface and covered with another transparent glass. After 1 minute, the spreadability of the gel was measured and recorded. Subsequently, 150 g was applied to the glass and left for another minute. The diameter of the spreadability was then calculated, with an ideal spreadability ranging between 5-7 cm. This test was done triplicate and reported as average and standard deviation [15].

**Adhesion test**

0.1 g of the gel was applied to a glass slide and then covered with another glass slide. Then, put a 500 g load on the glass slide for 5 minutes. The adhesion time was determined using a stopwatch. The glass slide was released when given a load of 80 g. The time required for the glass slide to be released was recorded [9].

**pH test**

The pH test involved weighing 1 gram of the sample and dissolving it in 10 mL of purified water, stirring until the mixture was homogeneous. The pH meter used a calibrated pH meter in the diluted sample [16].

**Viscosity test**

The test involved placing the gel into a container and attaching the appropriate spindle to a Brookfield viscometer, where measurements were done [17].

**Determination of the optimum formula of hand sanitizer gel**

After evaluating the physical and chemical properties of the gel, data analysis used the Design Expert software version 11 to determine the optimum formula [18].

**Hedonic test**

The hedonic test on the hand sanitizer preparation used 20 respondents aged 20 to 30. Respondents were in good health and did not have skin diseases. The hedonic test applied gel preparation to the respondent's palm. Then, the respondents filled out the questionnaire attached [19].

**Antibacterial testing**

All glassware and materials used were sterilized first, except for the soursop leaf extract and test microbes. The sterilization equipment used was an autoclave at 121 ˚C for 15-20 minutes. The test media preparation was by dissolving 38 grams of Muller Hinton Agar (MHA) in 1000 milliliters of purified water. The mixture was then stirred and heated on a hot plate. Furthermore, the MHA media was sterilized in an autoclave for 15 minutes at 121 ˚C. The media was then poured into a sterile Petri dish of 20 mL and carried out in the LAF [20]. The microorganisms used in this study were Staphylococcus aureus ATTC 6538. Preparation of test microbes was prepared aseptically on Muller Hilton Agar (MHA). The test samples for *Staphylococcus aureus* included a positive control (chloramphenicol), a negative control (formula without extract), and formula III. The control samples were soaked in the solution using disc paper for 5 minutes [21]. The treated control, soaked for 5 minutes, was placed on an agar medium containing Staphylococcus aureus culture and incubated at 37 °C for 18-24 hours. Monitored, and the inhibition zone was measured.

**Statistical analysis**

Data on the physical properties of the preparation obtained from this study were organoleptic, homogeneity, spreadability, pH, viscosity, and adhesion tests. The organoleptic and homogeneity data were descriptive. Spreadability, adhesion, and viscosity tests of each formula were analyzed using Design Expert Version 11. The Simplex Lattice Design (SLD) was chosen for its ability to optimize component combinations in mixture experiments, particularly when dealing with a fixed total amount of ingredients. While other formulation optimization methods were likely considered, SLD was selected because it efficiently explores the design space and allows for the identification of optimal formulations. Model validation was likely achieved by comparing the model's predictions with experimental results, as is common practice in model development. Then, hedonic tests were determined using questionnaires, and the results were descriptive.

## RESULTS

**Table 4.1** Phytochemical screening test of soursop leaf ethanol extract (*Annona muricata* L.).

| Sample | Alkaloids | Flavonoids | Saponins | Tannins |
|---|---|---|---|---|
| Soursop leaf extract | - | + | + | + |

Description: (+) Positive = compound present; (-) Negative = No Compound

**Table 4.2** Organoleptic observation results of hand sanitizer gel.

| Storage time (day) | Organoleptic | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Formula 1 | | | Formula 2 | | | Formula 3 | | |
| | Form | Color | Odor | Form | Color | Odor | Form | Color | Odor |
| 0 | HV | BG | GT | MV | BG | GT | LV | BG | GT |
| 1 | HV | BG | GT | MV | BG | GT | LV | BG | GT |
| 7 | HV | BG | GT | MV | BG | GT | LV | BG | GT |
| 14 | HV | BG | GT | MV | BG | GT | LV | BG | GT |
| 21 | HV | BG | GT | MV | BG | GT | LV | BG | GT |
| 28 | HV | BG | GT | MV | BG | GT | LV | BG | GT |

Notes: HV: High viscosity, BG: Blackish green, GT: Green tea, MV: Medium viscosity, LV: Low viscosity

**Table 4.3** Results of observations of hand sanitizer gel homogeneity.

| Storage time (day) | Homogeneity | | |
|---|---|---|---|
| | Formula 1 | Formula 2 | Formula 3 |
| 0 | Homogeneous | Homogeneous | Less homogeneous |
| 1 | Homogeneous | Homogeneous | Less homogeneous |
| 7 | Homogeneous | Homogeneous | Less homogeneous |
| 14 | Homogeneous | Homogeneous | Less homogeneous |
| 21 | Homogeneous | Homogeneous | Less homogeneous |
| 28 | Homogeneous | Homogeneous | Less homogeneous |

**Table 4.4** Observation results of hand sanitizer gel spreadability.

| | Storage time (day) | Spreadability (cm ± SD) | | |
|---|---|---|---|---|
| | | Formula 1 | Formula 2 | Formula 3 |
| F1 | 0 | 5.17 ± 0.18 | 6.20 ± 0.005 | 6.29 ± 0.10 |
| | 1 | 4.91 ± 0.04 | 5.99 ± 0.004 | 6.20 ± 0.12 |
| F2 | 7 | 4.61 ± 0.10 | 5.51 ± 0.02 | 5.63 ± 0.13 |
| | 14 | 4.48 ± 0.05 | 5.35 ± 0.03 | 5.34 ± 0.04 |
| | 21 | 4.41 ± 0.05 | 4.99 ± 0.07 | 4.88 ± 0.10 |
| F3 | 28 | 4.20 ± 0.03 | 4.79 ± 0.10 | 4.45 ± 0.06 |
| | Average± SD | 4.63 ± 0.07 | 5.47 ± 0.04 | 5.46 ± 0.09 |

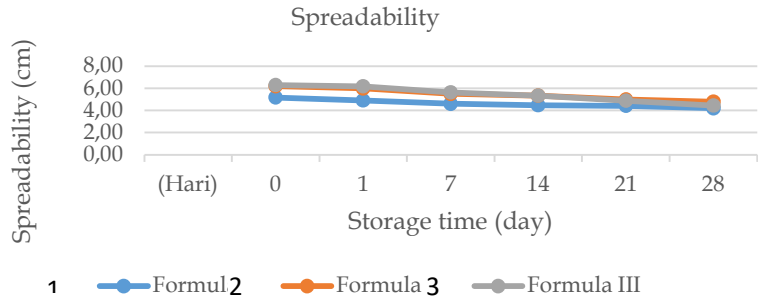



**Figure 4.1** Graph of spreadability of the hand sanitizer gels.

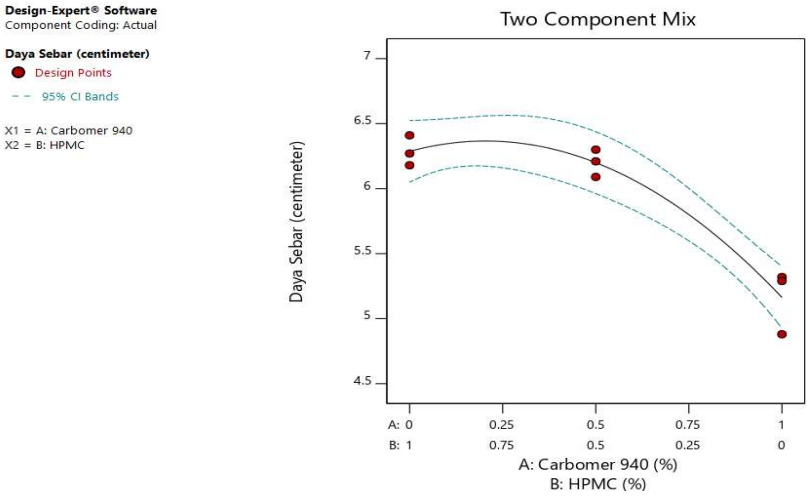



**Figure 4.2** Profile of spreadability of the hand sanitizer gel.

**Table 4.5** Observation results of hand sanitizer gel adhesion.

| Storage time (day) | Adhesion (Second ± SD) | | |
|---|---|---|---|
| | **Formula 1** | **Formula 2** | **Formula 3** |
| 0 | 18.33 ± 1.15 | 13.00 ± 1.00 | 10.00 ± 2.00 |
| 1 | 19.33 ± 1.53 | 13.33 ± 1.53 | 9.67 ± 1.53 |
| 7 | 19.67 ± 1.53 | 13.67 ± 2.08 | 9.33 ± 2.08 |
| 14 | 20.00 ± 1.00 | 14.33 ± 1.15 | 8.33 ± 2.52 |
| 21 | 25.00 ± 1.73 | 15.67 ± 1.53 | 6.67 ± 1.53 |
| 28 | 25.67 ± 1.53 | 18.33 ± 1.53 | 5.00 ± 2.00 |
| Average ± SD | 21.33 ± 1.41 | 14.72 ± 1.47 | 8.17 ± 1.94 |

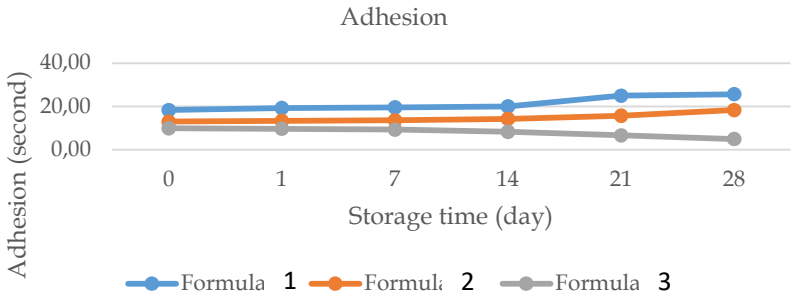



**Figure 4.3** Graph of the adhesion of the hand sanitizer gel.

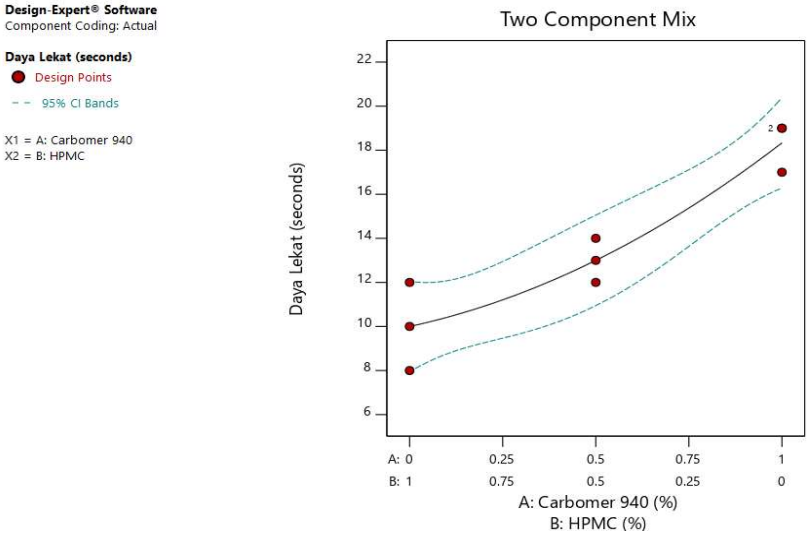



**Figure 4.4** Profile of adhesion test of hand sanitizer gel

**Table 4.6** Results of the pH of the hand sanitizer gel.

| Storage time (day) | pH | | |
|---|---|---|---|
| | Formula 1 | Formula 2 | Formula 3 |
| 0 | 5 | 5 | 5 |
| 1 | 5 | 5 | 5 |
| 7 | 5 | 5 | 5 |
| 14 | 5 | 5 | 5 |
| 21 | 5 | 5 | 5 |
| 28 | 5 | 5 | 5 |

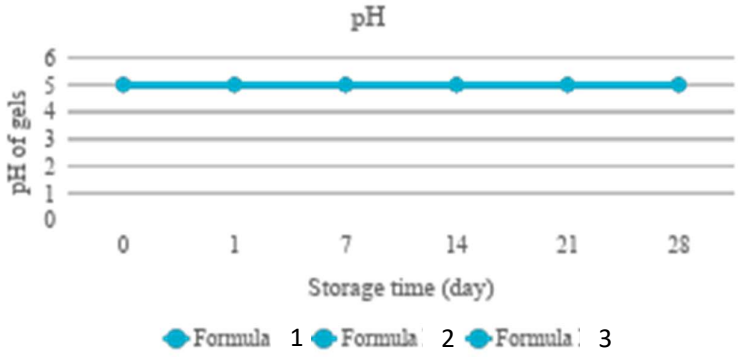



**Figure 4.5** Graph of pH gel hand sanitizer.

**Table 4.7** Observation results of hand sanitizer gel viscosity.

| Storage time (day) | Viscosity (cP ± SD) | | |
|---|---|---|---|
| | Formula 1 | Formula 2 | Formula 3 |
| 0 | 69.800±2.07 | 27.000±0.53 | 12.033±0.80 |
| 1 | 72.867±3.43 | 29.000±1.25 | 13.100±0.44 |
| 7 | 89.033±0.60 | 30.100±2.34 | 14.600±0.44 |
| 14 | 94.667±0.51 | 30.367±0.12 | 17.033±0.23 |
| 21 | 96.367±1.97 | 32.167±1.06 | 18.067±0.47 |
| 28 | 99.233±0.25 | 35.733±5.77 | 20.100±1.04 |
| Average ± SD | 86.994±1.47 | 30.844±1.84 | 15.822±0.57 |

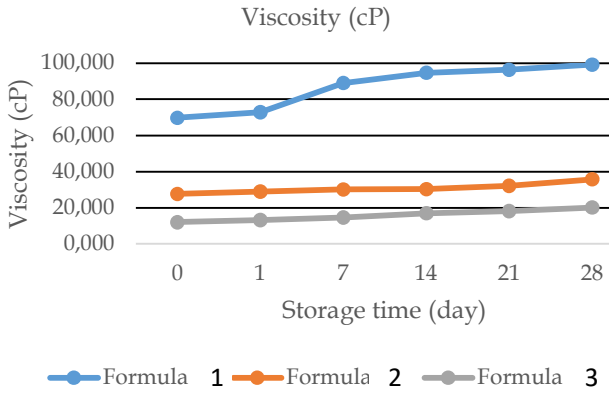



**Figure 4.6** The viscosity graph of the hand sanitizer gel.

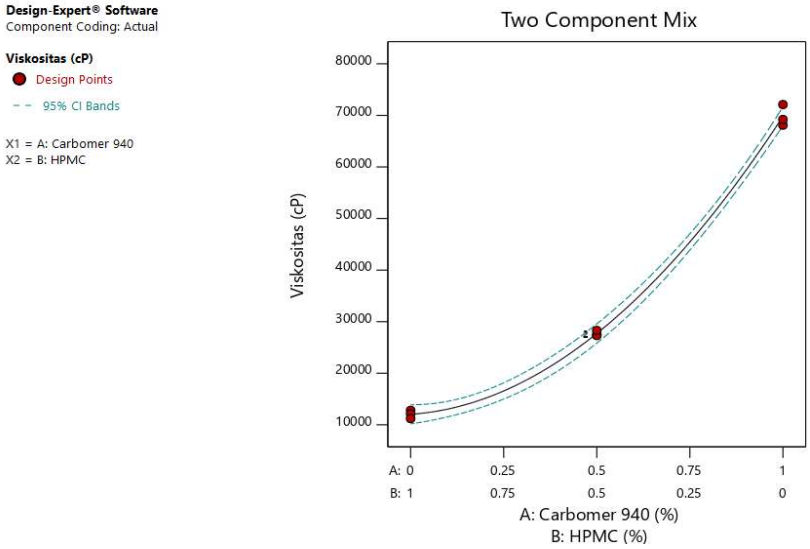



**Figure 4.7** Viscosity test profile of hand sanitizer gel.

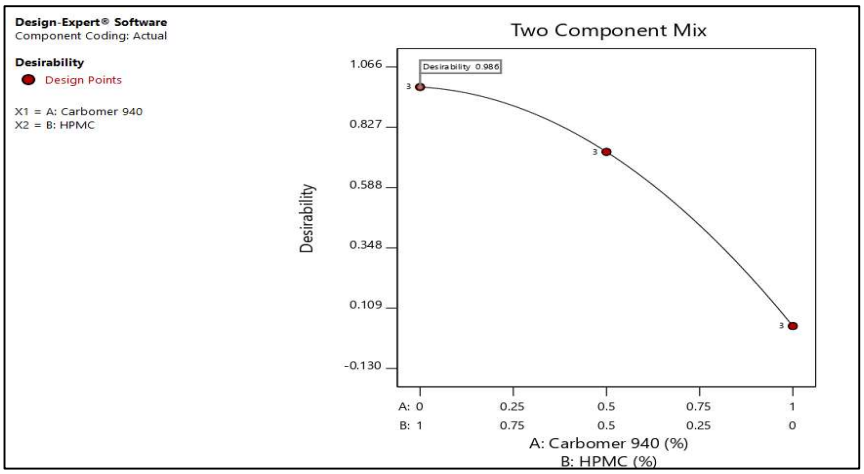



**Figure 4.8** Optimization profile of hand sanitizer gel formula.

**Table 4.8** Results of optimum formula testing with one-sample T-test.

| | Experiment results | Prediction results | Sig |
|---|---|---|---|
| Spreadability (cm) | 6.29 | 6.2867 | 0.965 |
| Adhesion (second) | 10.00 | 10 | 1.000 |
| Viscosity (cP) | 12033 | 12033.3 | 1.000 |

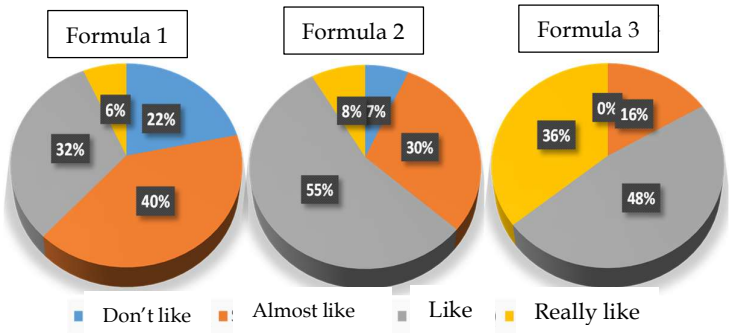



**Figure 4.9** Pie diagram of hedonic test with a combination of carbomer 940 and HPMC.

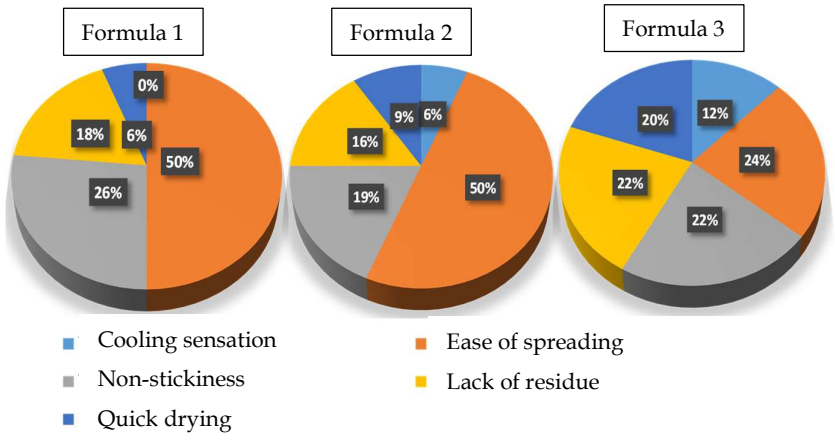
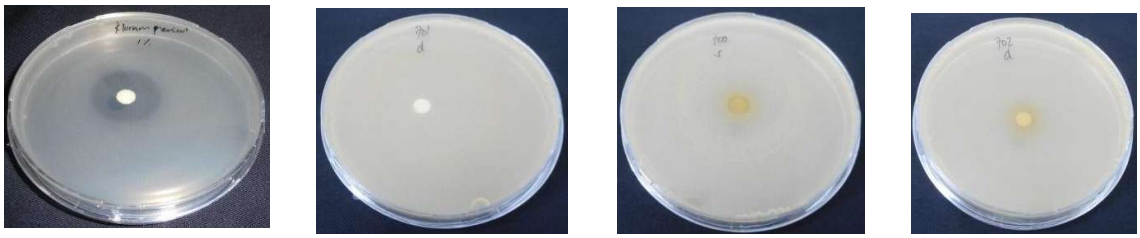



**Figure 4.10** Pie diagram of hedonic test characteristics of the hand sanitizer gels with a combination of carbomer 940 and HPMC.

**Table 4.9** Antibacterial activity test results.

| Samples | Microbial test *Staphylococcus aureus* | Inhibition (mm) |
|---|---|---|
| Positive control | + | 22.60 |
| Soursop leaf extract | - | 0 |
| Basis gel *hand sanitizer* | - | 0 |
| Formula 3 | - | 0 |

Description: (+) There is inhibition, (-) No inhibition



Positive control | Negative control | Soursop extract | Formula 3

**Figure 4.11** Antibacterial activity test results.

## ▪ DISCUSSION

### Extraction of soursop leaf samples

The soursop leaves were from Balitro, an Indonesian Spices Medicinal and Aromatic Plants Instrument Standard Testing Institute. The plant determination was at the Plant Conservation Center of the Botanical Gardens-LIPI, Bogor. The plant determination aimed to prove that the plant used in the study was correct. The determination results showed that the sample used in this study was Annona muricata L from the Annonaceae family. In this study, the sample used was soursop leaves. The samples used are green leaves. The leaves are 14.7-15.5 cm x 6.3 - 6.4 cm in size. Then, the sample selection was to separate the samples from dirt or foreign materials. From the sorting, 12 kg of wet soursop leaves were obtained, washed clean using running water, and cut into small pieces to dry quickly. Leaf drying used an oven at 40$^{o}$C for 24 hours. The temperature was not too high to prevent the destruction of compounds that could not withstand high heat. The dried leaves were crushed into powder using a blender to reduce the size of the soursop leaf particles. According to Ningsih et al. (2016) [22], the smaller the size of the material used, the wider the contact area between the material and the solvent, making it easier to extract active compounds. The sample preparation resulted in 3 kg of soursop leaf powder.

The soursop leaf powder underwent maceration using ethanol as the solvent. This technique is effective in producing large quantities of extracts while preserving compounds that are sensitive to heat. [23]. The maceration process was carried out at room temperature to minimize damage or degradation of metabolites [24]. During the maceration process, stirring was carried out occasionally every day to avoid the accumulation of powder that could make it difficult for the solvent to penetrate the active ingredients and compounds [22]. The obtained extract was a thick blackish-green of 341.6 g. The yield of the thick extract was 11.38%. The extract obtained was stored in a glass container and protected from sunlight.

#### *Loss on drying of extract*

Loss on drying (LOD) was the remaining substance after drying at 105oC for 30 minutes or until a constant weight, expressed in percent. LOD aimed to provide a maximum limit on the amount of compound lost in the drying process (Ministry of Health of the Republic of Indonesia, 2000). The constant weights were obtained after three times drying with an ovens. In this study, the LOD was 5.96%, indicating that the extract did not lose much of the compound that evaporated during heating. The test results met the requirements for drying loss weight, which was <10% [10].

*Phytochemical screening test*

The phytochemical screening test determined the presence of secondary metabolite compounds in soursop leaves. The screening test of phytochemical content used a test tube that reacted the sample with a reagent solution. The test was to determine the presence of alkaloid, flavonoid, saponin, and tannin compounds.

Testing of alkaloid compounds of soursop leaf ethanol extract used Mayer and Dragendorff reagents. A positive result was if a white precipitate formed when Mayer's reagent or a brick-red precipitate formed when Dragendorff's reagent was added [11]. Alkaloids have nitrogen atoms and free electron pairs to form coordinate covalent bonds with metal ions [25]. In this test, alkaloids are present using Dragendorff's reagent. The nitrogen atoms in the alkaloids formed coordinate covalent bonds with the metal ion K+ [26]. If the alkaloids test used Mayer's reagent, the results did not form a white precipitate. The nitrogen in alkaloids did not react with the metal ion K+ from potassium tetraiodomercurate (II), so a potassium-alkaloid complex that could precipitate unhappened. According to the Indonesian Ministry of Health (1995) [11], in testing alkaloid compounds, it was said to be positive if the reaction that formed a precipitate used at least two groups of experimental responses, so it meant that the alkaloid compound in soursop leaf extract had negative results. Testing of flavonoid compounds used magnesium metal (Mg) and concentrated HCl. Magnesium and HCl reduced the benzopyrone core in the flavonoid structure [27].

The formation of a red, yellow, or orange solution shows positive results [12]. The results of this study showed positive results in the formation of a red solution. Saponin compound testing used hot purified water in a test tube shaken vigorously for 10 seconds. If the foam appeared, one drop of 2 N HCl addition did not cause the foam to disappear. The results of this study showed that there was foam as high as 1 cm, which did not disappear for ± 10 minutes. The samples of soursop leaf extract contained saponin compounds. The foam formation is due to saponin compounds' ability to reduce the surface tension of water. Saponins had large molecules containing hydrophilic and lipophilic groups like soap. In water, saponin molecules are aligned vertically on their surface with lipophilic groups away from water. The adsorption of saponin molecules on the water surface could decrease the surface tension of the water, which could cause foam [28]. In tannin testing, FeCl3 reagent addition and positive results were proven by a blackish-blue or blackish-green solution [13]. The tannin test in this study revealed a blackish-green solution upon the 1% FeCl3 addition, indicating that the ethanol extract of soursop leaves contains tannin compounds.

Phytochemical tests using FeCl3 were to determine whether the sample consisted of phenol groups. The addition of FeCl3 resulted in a blackish-green or dark blue color, indicating the presence of phenol groups. Therefore, if the phytochemical test with FeCl3 was positive, it suggested the presence of phenol compounds in the sample, including tannin, as tannin is a polyphenol compound. The formation of a blackish-green or ink-blue color in the extract after FeCl3 addition, then tannin would form a complex compound with Fe3+ ions [27]. Based on phytochemical screening tests, ethanol extract of soursop leaves contained flavonoid, saponin, and tannin compounds.

**Hand sanitizer gel preparation**

This study made 3 (three) hand sanitizer gel formulas with different gel base concentrations, all using soursop leaf extract with a concentration of 12%. The gel preparation consisted of active substances and excipients. The active substance used in this study was soursop leaf ethanol extract as an antibacterial. Excipients were additional substances needed because they had a key function in preparation. The excipients used in this study were carbomer 940 base, HPMC, methylparaben, EDTA, glycerin, pure water, green tea fragrance, and TEA. The preparation of hand sanitizer gel used a combination of carbomer 940 and HPMC gel bases. Carbomer 940 and HPMC were selected because they could produce gel preparations with sufficient viscosity, are soluble in water, produce clear gels, and have harmless and non-toxic properties. These polymers interact synergisly by giving positive results.

The physical evaluation of the hand sanitizer gel included organoleptic observations (shape, color, and odor), homogeneity, spreadability, adhesiveness, pH, and viscosity. The evaluation was done on days 0, 1, 7,

14, 21, and 28 to determine whether the hand sanitizer gel was stable and met the requirements. The evaluation results of the hand sanitizer gel are as follows

***Organoleptic observation***

Organoleptic testing was to show the quality of the hand sanitizer gel by using five senses to observe the shape, color, and odor. The organoleptic tests of the hand sanitizer gels include shape, color, and odor. Then, the hand sanitizer gels were stored and evaluated for 28 days, and there were no changes in all formulas. Each formula had a different shape due to differences in the concentration of the gelling agent used. The higher the concentration of the gelling agent used, the thicker the resulting preparation. Formula 1 showed that the gel was very viscose, while Formula 2 was viscose, and Formula 3 was slightly viscose. Observations of the color of the three formulas showed a blackish-green color and smelled of green tea. The results of observations of the color and smell of the gel did not change from the first day to the 28th day. The conclusion was that the gel was stable during storage.

***Homogeneity observation***

Homogeneity testing was to observe whether the gel preparation made was homogeneous. The homogeneity results are in Table 4.3.

The results showed that Formula 1 and 2 were homogeneous and obtained because there were no coarse grains. For Formula 3, the results obtained were less homogeneous, and poor stirring could be the cause during manufacture. According to Purwaningsih et al. (2014), homogeneity could be affected by mixing techniques and preparation. [29] The results showed that Formula 1 and 2 were homogeneous, and after storage for 28 days, the gel preparation did not change.

***Spreadability***

Spreadability is the ability of a gel preparation to spread on the skin and is one of the key properties in semisolid preparations because it can be related to the ease of use and removal of the preparation from the packaging container. The greater the value of the spreadability of a gel preparation, the better its spreading ability, and vice versa. If the value of a preparation's spreadability is small, its spreading ability will be worse (Wulandari, 2015). The results of the spreadability measurement are in Table 4.4.

The parameters of good gel spreadability range from 5-7 cm. The results above showed that the gel spreadability met the requirements of Formula 2 and 3. This gel preparation was highly consistent, making it challenging to spread and flow. The longer the storage, the smaller the gel spreadability will be, and small spreadability could be caused by the water content in the gel preparation evaporating so that the preparation becomes viscose. The gel preparations with low viscosity could produce a larger spreadability diameter because they were easy to spread or flow.

The results of the gel spreadability test obtained the equation coefficient from the Simplex Lattice Design as follows: Y = 5.16 X1 + 6.29 X2 + 1.9 X1X2

Where: Y = spreadability (cm); X1 = proportion of carbomer 940 100%; X2 = proportion of HPMC 100%; X1X2 = proportion of carbomer 940: HPMC (50% : 50%). According to the equation above, the component had a positive coefficient value, indicating that the component could increase the spreadability response. The proportion of HPMC 100% (coefficient value 6.29) had the highest effect on raising the spreadability compared to the addition of the carbomer 940 component (coefficient value 5.16) and a mixture of HPMC and carbomer 940 (coefficient value 1.90).

***Observation of adhesion***

Adhesion testing plays a role in the effectiveness of a preparation in providing an effect. The longer the adhesion of a preparation, the greater the pharmacological activity produced. In this study, the test results are in Table 5.5.

Based on the study, the adhesion of the hand sanitizer gel during storage for Formula 1 and 2 increased, whereas the adhesion of Formula 3 decreased. According to Nurwulan & Taufani (2017) [30], good adhesion should be more than 0.07 minutes or 4 seconds. The adhesion of a preparation is directly proportional to viscosity. The higher the viscosity value, the higher the adhesion. The longer the gel adheres to the skin, the more effective it will be because the absorption of the active substance increases [31]. From the observation data, the adhesion of the hand sanitizer gels met the requirements.

The results of the gel adhesion test obtained the equation coefficient from the Simplex Lattice Design as follows: Y = 18.33 X1 + 10.00 X2 - 4.64 X1X2, where: Y = adhesive power (seconds); X1 = proportion of carbomer 940 100%; X2 = proportion of HPMC 100%; X1X2 = proportion of carbomer 940: HPMC 50%: 50%. Based on the results of the equation above, the components had positive and negative coefficient values, which meant they could increase and decrease the adhesion. The proportion of carbomer 940 (coefficient value 18.33) had the highest effect on raising adhesion compared to the addition of the HPMC component (coefficient value 10.00). The coefficient value of the mixture proportion between carbomer 940 and HPMC was negative. This result could cause a decrease in adhesion.

***pH observation***

The pH test was to determine the acidity level of the hand sanitizer gel to ensure that it did not cause skin irritation. The pH was measured using a calibrated pH meter immersed in the diluted gel sample. Table 4.5 shows the results of the pH measurement.

Based on observations for 28 days of storage, the hand sanitizer gel preparation did not experience any changes in pH. The pH value produced from each formula was safe because it was within the criteria for skin pH (4.5-6.5) [32]. The pH results did not obtain an equation in optimization because the pH obtained already had a value that met the standard, namely 5.

***Viscosity observation***

The viscosity measurement of hand sanitizer gel preparations used a Brookfield Viscometer with spindles of 64 and 6 rpm. From the storage results for each formula from day 0 to day 28, they had different values and increased. The good viscosity value of the gel preparation was 20,000-40,000 cP [33]. From the data obtained, the viscosity of the gel preparation that met the requirements was Formula 2 and 3. The viscosity could be affected by the concentration of the gelling agent used. The greater the concentration of the gelling agent used, the thicker the resulting preparation. The results of the viscosity measurement are in Table 4.7.

The results of the gel viscosity test obtained the equation coefficients from the Simplex Lattice Design as follows: Y = 69.800 X1 + 12.033 X2 – 52.866 X1X2, where: Y = viscosity (cP); X1 = proportion of Carbomer 940 100%; X2 = proportion of HPMC 100%; X1X2 = proportion of carbomer 940: HPMC 50%: 50%. Based on the equation above, the components had positive and negative coefficient values, which meant they could increase and decrease viscosity. The proportion of carbomer 940 (coefficient value 69.800) has the highest effect on increasing viscosity compared to the addition of HPMC components (coefficient value 12.033). The negative coefficient value of the mixture proportion between carbomer 940 and HPMC can reduce viscosity.

**Optimization of hand sanitizer gel formula of ethanol leaf extract of soursop (*Annona muricata* L.)**

The hand sanitizer gel formulas were optimized by showing the desired physical properties. The data was obtained from the physical evaluation of the gel preparation and analyzed using design expert software version 11. Figure 4.8. showed the optimization results.

The formula optimization profile in the figure above showed that the formula with a composition ratio of carbomer 940: HPMC (0%: 1%) had a desirability value of 0.986. According to Suryani et al. (2017) [34], the maximum desirability value was one, so if the desirability value approaches one, the value is better. This result showed that the desirability value obtained in this study is as expected. The hand sanitizer gel made from soursop leaf ethanol extract achieved the highest desirability value and was verified using a one-sample t-test for spreadability, adhesion, and viscosity. This verification determined the difference between the experimental results and the predicted results. The test results are in Table 4.9.

Table 4.8 shows the optimum formula with a significant value of more than 0.05. The method used was valid, as there was no significant difference between the experimental results and the predictions.

**Hedonic testing**

Hedonic testing was to determine the preference level for the hand sanitizer gel of soursop leaf ethanol extract. This test involved 20 respondents. The test parameters included organoleptic, such as shape, color, odor, and specific properties of the gel. Each respondent was given a questionnaire by filling in the preference level based on the scale and characteristics of the preparation. Each respondent received three hand sanitizer gels. Based on the organoleptic hedonic test of the three preferred formulas, Formula 3 contained a mix of carbomer 940: HPMC (0%: 1%). The organoleptic test provided the following results:

Hedonic testing evaluated hand sanitizer characteristics such as a cooling sensation, ease of spreading, non-stickiness, lack of residue, and quick drying. According to the test results, Formula 3 achieved the highest score of 81 (Figure 4.10).

Based on the results of the hedonic characteristics, the best formula was Formula 3 of carbomer: HPMC (0%: 1%). After respondents tested the hand sanitizer gels, they provided opinions on the hand sanitizer formula. Fifteen respondents gave an agreed response, while five disagreed. Respondents who agreed gave several comments. For example, the hand sanitizer gel contained a refreshing aroma, Formula 3 had a shape close to hand sanitizer products on the market, the preparation was not sticky when used, and it dried faster than Formula 1 and 2. Five respondents who disagreed commented that the hand sanitizer gel had a less attractive color, left marks when used, and was sticky.

**Antibacterial testing**

Antibacterial activity testing determined the presence of resistance to Staphylococcus aureus bacteria. The antibacterial activity test of soursop leaf ethanol extract (Annona muricata L.) did not show any inhibition as was done by Widyawati (2017) [2], which showed that soursop leaf ethanol extract had antibacterial activity at a concentration of 12% with an inhibition zone of 22 mm. However, the results showing no inhibition were likely due to many possibilities, such as the low extract concentrations or species variation of natural sources. This study was in line with research conducted by Yovitasari et al. (2018) [23] that soursop leaf extract has inhibitory zone at concentrations of 25%, 50%, 75%, and 100% with inhibitory power of 4 mm, 5.3 mm, 8.9 mm, and 12.4 mm.

## CONCLUSION

This study can produce the soursop extract and hand sanitizer gels. The hand sanitizer gels are produced using a combination of carbomer 940 and HPMC. The gel formulas can be optimized using a Simplex Lattice Design experimental method.

The results of physical properties tests (spreadability, adhesion, and viscosity) were analyzed using Design Expert 11. The results showed that carbomer 940 and HPMC could increase viscosity and adhesion but could decrease spreadability. Ethanol extract of soursop leaves (*Annona muricata* L.) could be formulated and optimized into hand sanitizer gels. The optimum formula contained carbomer 940 0% and HPMC 1%, obtained in Formula 3 with a desirability value of 0.986.

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## REFERENCES

[1] W. Arozal, M. Louisa, and V. Soetikno. Selected Indonesian medicinal plants for the management of metabolic syndrome: Molecular basis and recent studies. Front Cardiovasc Med. 2020; 6(7): 82. https://doi.org/10.3389/fcvm.2020.00082. PMID: 32435657; PMCID: PMC7218133.

[2] L. Mardiana, and J. Ratnasari. *Ramuan dan Khasiat Sirsak*. Jakarta: Penebar Swadaya, 2014.

[3] L.Widyawati, B.A.A. Mustariani, and E. Purmafitriah. Formulatıon of gel hand sanıtızer ethanol extract of soursop leaf (*Annona murıcata* Linn) as antıbacterıal to *Staphylococcus aureus*. J Farmasetis. 2017; 6(2): 47-57. https://doi.org/10.32583/farmasetis.v6i2.274

[4] E. Lindawati, N. Lestarie, E. Nurlaela, M. Rival, and S. Maryati. Inovasi "Kewangi" sebagai gel antiseptik alami dari minyak atsiri Kemangi (*Ocimum canum*). Laporan Akhir-PKM Penelitian. Bogor: IPB, 2014.

[5] M. Shu. Formulasi sediaan gel hand sanitizer dengan bahan aktif triklosan 0,5% dan 1%. Calyptra. 2013; 2(1): 1–14.

[6] J.I. Wijaya. Formulasi sediaan gel hand sanitizer dengan bahan aktif triklosan 1,5% dan 2%. Calyptra. 2013; 2(1), 1–14.

[7] F. Firmansyah, H. Kholifah, and L. Chabib. Formulasi gel hand sanitizer ekstrak buah Belimbing Wuluh dengan variasi karbopol 940 dan HPMC. J Islamic Pharm. 2022; 7(1): 69-73. https://doi.org/10.18860/jip.v7i1.13839

[8] N. Nahdhia, M.A.S. Rijal, E. Hendradi, and R.T. Widodo. (2024). Application of the Simplex Lattice Design Method to determine the optimal formula of diclofenac sodium nanoemulsion. JFIKI. 2024; 11(2): 137-146. https://doi.org/10.20473/jfiki. v11i12024.137-146

[9] V. Saraung. Pengaruh varıası babıs karbopol dan hpmc pada formulası gel ekstrak etanol daun Tapak Kuda (*Ipomoea pes-caprae* (L.) R. Br. dan ujı aktıvıtas antıbakterı terhadap *Staphylococcus aureus*. Pharmacon. 2018; 7(3). https://doi.org/10.35799/pha.7.2018.20452

[10] Depkes RI. Farmakope Herbal Indonesia (I). Jakarta: Depkes RI , 2000.

[11] Depkes RI. Materia Medika Indonesia (V–VI). Jakarta: Depkes RI, 1995.

[12] J.B. Harborne. Metode Fitokimia. Bandung: Institut Teknologi Bandung, 1987. (Translation)

[13] E. Kusumawati, A. Apriliana, and R. Yulia. Kemampuan antibakteri ekstrak etanol daun Nangka (*Atrocarpus heterophyllus* Lam.) terhadap *Escherichia coli*. J Sains Kes. 2017; 1(7): 327–332. https://doi.org/10.25026/jsk.v1i7.51

[14] L. Nurdianti, S.F. Azzahra, and N. Aji. Pengembangan formulasi sediaan gel rambut antiketombe ekstrak daun Pandan Wangi (*Pandanus amaryllifolius* Roxb.) dengan menggunakan viscolam sebagai gelling agent dan uji aktivitasnya terhadap jamur Pityrosporum ovale. JKBTH. 2017; 17(2): 456–467.

[15] J. Adnan. Formulasi gel ekstrak daun Beluntas ( *Pluceaindica less* ) dengan Na-CMC sebagai basis gel. J Pharm Sci Herb Technol. 2017; 1(1): 41–44.

[16] M.S. Lateh. "Formulasi sediaan gel tangan sanitizer ekstrak etanol buah Asam Gelugur (*Garcinia atroviridis* Griff. et Anders) sebagai antibakteri terhadap Staphylococcus aureus," M.S. thesis, Univ. Muh. Surakarta, Surakarta, Jawa Tengah, 2015.

[17] T. Prayoga, I. Agustina, N. Lisnawati, R. Robby, and N. Hidayah. Formulası gel hand sanıtızer ekstrak daun Belımbıng Wuluh (*Averrhoa bilimbi* L.). JIIS. 2024; 9(1): 209-17. https://doi.org/10.36387/jiis.v9i1.1860

[18] H.F. Asyharı, K.B. Cabral, and E.R. Wıkantyasnıng. Optimization of Soursop (*Annona muricata* L.) leaf extract in nanoemulgel and antiacnes activity test against *Propionibacterium acnes, Staphylococcus aureus, Staphylococcus epidermidis* bacteria. Pharmacon. 2023; 20(2): 216-225. https://doi.org/10.23917/pharmacon.v20i2.23308.

[19] D. Saryanti, and I.N. Zulfa. Optimasi karbopol dan gliserol sebagai basis gel antiseptik tangan ekstrak etanol daun Ceremai (*Phyllantus acidus* (L.) skeels) dengan Metode Simplex Lattice Design. J Pharm Sci Clin Res. 2017; 02(01): 35–43. https://doi.org/10.20961/jpscr.v2i01.5238

[20] F.L. Mahmudah, and S. Atun. Uji aktivitas antibakteri dari ekstrak etanol Temu Kunci (*Boesenbergia pandurata*) terhadap bakteri Streptococcus mutans. JPS. 2017; 22(1): 59–66.

[21] O.S. Marsono, T.E. Susilorini, and P. Surjowardojo. Pengaruh lama penyimpanan dekok daun Sirih Hijau (*Piper betle* L.) terhadap aktivitas daya hambat bakteri *Streptococcus agalactiae* penyebab matitis pada sapi perah. JITST. 2017; 12(1): 47–60.

[22] D.R. Ningsih, Zusfahair, and D. Kartika. Identifikasi senyawa metabolit sekunder serta uji aktivitas ekstrak daun Sirsak sebagai antibakteri. J Mol. 2016; 11 (1): 101-111.

[22] D. Yovitasari, A. Retnaningsih, and V. Elsyana. Uji daya hambat ekstrak daun Sirsak (*Annona muricata* L.) terhadap pertumbuhan bakteri *Staphylococcus aureus* dan *Pseudomonasa eruginosa* dengan metode difusi agar. J Farm Malahayati. 2018; 1(1): 1–4.

[23] E. Hanani. Analisis Fitokimia. Jakarta: Buku Kedokteran EGC, 2017.

[24] Â. Liberal, A.K. Molina, C. Pereira, M.I. Dias, I.C.F.R. Ferreira, and L. Barros. Chapter 4 - Solid-liquid extraction of polyphenols. Technologies to Recover Polyphenols from AgroFood By-products and Wastes. Academic Press: Elsevier, 2022, p. 73-112.

[25] D. Kumaradewi, W. Subaidah, D. Andayani, and A. Al-Mokaram. Phytochemical Screening and Activity Test of Antioxidant Ethanol Extract of Buni Leaves (Antidesma bunius L. Spreng) Using DPPH Method. JPP IPA. 2021; 7(2): 275-280. https://doi.org/10.29303/jppipa.v7i2.675

[26] S.D. Marliana, V. Suryanti, and Suryono. Skrining fitokimia dan analisis kromatografi lapis tipis komponen kimia buah Labu Siam (*Sechium edule* Jachq.Swartz) dalam ekstrak etanol. Biofarmasi. 2005; 3(1): 26–31.

[27] E. Ergina, S. Nuryanti, and I.D. Pursitasari. Uji kualitatif senyawa metabolit sekunder pada daun Palado (*Agave angustifolia* ) yang diekstraksi dengan pelarut air dan etanol. J Akad Kim. 2014; 3(3): 165–172.

[28] Y.S. Puspariani, "Isolasi dan identifikasi saponin pada kecambah Kedelai (*Glycine max* L.)," Thesis, Chemistry Dept., Faculty of Pharmacy, Universitas Sanata Dharma, Yogyakarta, 2007.

[29] S. Purwaningsih, E. Salamah, and T.A. Budiarti. Formulasi skin lotion dengan penambahan karagenan dan antioksidan alami dari *Rhizophora mucronata* Lamk . Akuatika. 2014; 5(1): 55–62.

[30] A. Nurwulan, and I.P. Taufani. Optimasi basis gel ekstrak etanol daun Sirsak (*Annona muricata* L.) berbasis kombinasi HPMC dan karbopol sebagai anti jerawat menggunakan aplikasi faktorial desain. NPKTI. Universitas Muhammadiyah Yogyakarta, 2017. https://www.scribd.com/document/425188752/12-NASKAH-PUBLIKASI.

[31] N. Octavia). Formulasi sediaan gel hand sanitizer minyak atsiri Pala (*Myristica fragrans* Houtt.): Uji stabilitas fisik dan uji aktivitas antibakteri terhadap bakteri *Staphylococcus aureus*. Universitas Muhammadiyah Surakarta, 2016. https://eprints.ums.ac.id/45035/1/Naskah%20Publikasi.pdf

[32] R.I. Tranggono, and F. Latifah. Buku Pegangan Ilmu Pengetahuan Kosmetik. Jakarta: Gramedia Pustaka Utama, 2007.

[33] Y. Sukawaty, A. Apriliana, and H. Warnida. Formula dan evaluasi gel pembersih tangan ekstrak Bawang Tiwai (*Eleutherine bulbosa* ( Mill .) Urb). Jurnal Ilmiah Manuntung. 2017; 3(1): 77–82. https://doi.org/10.51352/jim.v3i1.94

[34] S. Suryani, A. Nafisah, and S. Mana'an, S. (2017). Antioxidants gel formula optimation of Bligo fruit ethanolic extract (*Benincasa hispida*) by Simplex Lattice Design (SLD) method. Galenika J Pharm. 2017; 3(2): 150-156. https://doi.org/10.22487/j24428744.0.v0.i0.8815.