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Formulation of patchouli oil spray gel (*Pogostemon cablin* Benth) and irritation test in rabbit

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ABSTRACT

Patchouli plant (*Pogostemon cablin* Benth) is one of the essential oil-producing plants widely used in perfume, cosmetic, aromatherapy, antiseptic, antibacterial, and antifungal industries. The essential oil derived from patchouli leaves contains terpenoid compounds with antibacterial and antifungal activity. This study aims to determine the optimum formulation of patchouli oil spray gel as a wound antiseptic and to observe the irritation test of the samples. The spray gel formulations of patchouli oil were prepared in various concentrations i.e. 5%, 7.5%, and 10% (v/v), solved in aquadest with the addition of carbopol 940 as the gelating agent. The spray gels were tested to determine their physical characteristics, including the organoleptic test, homogeneity test, pH, adhesive dispersion test, viscosity test, and spray pattern test. The results showed that the patchouli oil spray gels were yellowish-white and had a distinctive odor and homogeneous texture with a pH 5. They all had adhesive dispersion, and the optimum viscosity of the spray gel was found at a 7.5% (v/v) concentration of patchouli oil. The irritation test was carried out using the patch test method with a score of erythema and edema. The results showed that all three formulas very mild irritation.

Keywords: *pogestemon oil, erythema, edema, topical, dermal irritation*

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INTRODUCTION

Indonesia's natural resources are gifted with plentiful commodities that are the potential to be utilized. The Indonesian natural biodiversity is estimated to reach around 40,000 species of plants, and one of them is patchouli (*Pogostemon cablin* Benth). Patchouli belongs to the Labiatea family, which contains saponins and flavonoids, tannins, glycosides, terpenoids, and steroids. In addition, this plant is a tropical shrub that produces essential oils commonly known as patchouli oil. This plant is commonly used for an essential oil that is extracted from its leaves. In industry, patchouli oil is widely used as a blending agent and aroma binder. Patchouli oil is obtained from the distillation of the leaves and stems. The main content of patchouli oil is patchouli alcohol (40-50%) which is used as raw material, mixing material, and fixative (binder) in perfume, cosmetic, and pharmaceutical industries. The main components of patchouli oil consist of sesquiterpenes and patchouli alcohol. Patchouli essential oil has been used as raw material in the perfume industry, lotion, soap, shampoo, and cosmetics (Ermaya et al., 2019). The saponins and tannins in patchouli essential oil may act as antibacterial compounds. In addition, saponins are also known to encourage the growth of collagen tissue. Previous studies have shown that patchouli oil has several pharmacological activities, such as antibacterial properties (Kongkathip et al., 2009). Antibacterial activity in inhibiting (bacteriostatic) or killing bacteria (bactericidal), especially bacteria that are harmful to humans (Fauzi et al., 2017). Research proved that patchouli leaf essential oil has antibacterial agent, *S. aureus and E.colli* (Dzakwan, 2012). The main components of essential oils from terpenoids in a plant may act to damage bacterial cell membranes by binding to the enzyme proteins and damaging the cell membranes that inhibit the growth of bacterial cells. The high content of patchouli alcohol shows an ability to fight the activity of bacteria such as *Bacillus subtilis* and *Staphylococcus aureus*. The use of patchouli oil has also been developed in a spray gel preparation that is able to accelerate wound healing. (Nonci et al., 2017) stated that patchouli oil has the ability to heal cuts in mice (*Mus musculus*) at a concentration of 50%, which shows the best activity in wound healing. Recovery in cut wounds is due to the presence of collagen tissue, which can accelerate wound healing. The wound healing process is generally divided into several phases, each of which is interrelated, namely the inflammatory, proliferative, and maturation phases. Collagen is a key component in this phase of wound healing. Immediately after tissue trauma, exposure of fibrillar collagen to the blood generate platelet aggregation and activation, which also releasing chemotactic factors that initiate the wound healing process. Collagen fragments release leukocytic collagenase to attract fibroblasts to the traumatized tissue. Furthermore, collagen becomes the foundation for the newly formed extracellular matrix (Zulkarnain et al., 2013). The selection of concentrations was carried out by trial and error to see how effective the patchouli oil was with only small concentrations.

The use of essential oils preparation could efficiently promote wound healing. Modification of preparation is important to make it easy in the application process. The preparation can be in gel spray form with the aim of simplifying the application process using a spray applicator without applying directly by hand. Gels are semi-solid systems consisting of either suspension made up of small inorganic particles or large organic molecules interpenetrated by a liquid. The consistency of the gel has to be adjusted as good as possible so the gel is easy to apply.

Preparation of sample in a spray gel aimed as an alternative antiseptic for wound healing. Preparation of sample spray in a gel form can last a long time when applied to the skin because of the gelling agent (Kamishita et al., 1992). In addition, spray gel is one of the most suitable topical preparation for inflammation in wound healing. This spray preparation is more practical for use and also is safer by the lower microorganism contamination since it is sprayed without hand contact like other tropical preparations. In this investigation, the spray gel preparations were tested to determine their physical properties, which these physical properties could affect the achievement of the pharmacological effect of the preparation (Anindhita & Oktaviani, 2020).

Terpenoid components in patchouli leaves can react with porin (transmembrane protein) on the outer membrane of the bacterial cell wall, forming strong polymer bonds, resulting in damage to the porin. The damage of porin–which is the entrance and exit routes of compounds, will reduce the

permeability of the bacterial cell wall and leading scarcity of bacterial nutrients, resulting in inhibition of bacterial growth or even leading to dead. Based on a comparative study between *Escherichia coli* and *Staphylococcus aureus* with the same concentration (50% concentration, each), it has a larger inhibition diameter against *Staphylococcus aureus* than *Escherichia coli*. This indicates that patchouli essential oil is more effective or potent against gram-positive bacteria like *Staphylococcus aureus* but less effective for gram-negative bacteria such as *Escherichia coli*. This might be due to the peptidoglycan layer in gram positive bacteria.

The patchouli oil spray gel is intended to be applied to the skin, so it is necessary to do an irritation test to ensure its safety. The patchouli alcohol potentially allows irritation in repeated use. Irritation is one of the adverse reactions that occur on the skin, which can be caused by various factors, including the duration of administration, the area of administration, the level of penetration, and the toxicity of the substance applied (Rowe et al., 2019). The irritation appearance emerges after several times applications of the sample, which is recognized by the presence of several symptoms such as dry skin, cracking, pain, and bleeding. Irritation of the skin is recognized by erythema and edema. Erythema or redness occurs due to the dilatation of blood vessels in the irritated area, while edema occurs when the frozen plasma enlarges in the injured area (Kamishita et al., 1992). Essential oils may cause uncomfortable effects when used directly (Wasitaatmadja, 1997). The discomfort that might occur is irritation can be recognized by redness and swelling. Therefore, this study was conducted to determine the physical properties of patchouli oil spray gel (Patchouli alcohol) and conduct the irritation test.

MATERIALS AND METHODS

Materials

The ingredients used in spray gel formulations were patchouli oil (Darjeeling), carbopol 940, triethanolamine (TEA), glycerin, tween 80, methyl paraben, propyl paraben, alpha tocopherol, span 20, which all purchased from PT. Brataco Indonesia and aquadest. While the ingredients for the irritation test included bioplacenton, acclimatized male rabbits, non-irritating bandages (Hypafic), sterile gauze (Onemed), anti-irritant markers (Triton), Rabbit (*Oryctolagus cuniculus*), that were obtained from a farm in Kendal, Central Java.

Methods

The research design performed is an experimental laboratory. It was conducted by modifying a spray gel formulation with various concentrations of patchhouli oil i.e 5%, 7.5%, and 10% (v/v) of the total volume of the spray gel. The spray gel preparation was divided into emulsion and emulgel phases. While the emulsion was also divided into two phases, namely the oil and aqueous phases. The aqueous phase was carried out by mixing tween 80 and aquadest that were heated until homogeneous. While the manufacture of the oil phase was done by mixing the patchouli oil, span 20, alpha tocopherol and stirring until homogeneously mixed (mixture 1). Next, the propylene glycol, nipagin, and nipasol were also mixed in a porcelain dish and stirred homogenously (mixture 2). The mixture 1 and 2 were then placed into a beaker glass and stirred using a magnetic stirrer at 1000 rpm for 5 min. The emulgel phase was conducted by weighing a 0.75 g of carbopol 940, TEA, glycerin, and aquadest using a measuring cup. At first, aquadest was boiled and placed into a container containing carbopol 940, then stirred until homogeneous. The glycerin was added little by little until mixed evenly into the solution, as well as the TEA. The addition of TEA allows to neutralize the acidity of carbopol, so that appearance of the gel preparation will be clear. Below are the formulations of the patchouli oil spray gel (Table 1).

Table 1. The formula of the patchouli oil spray gel

Contents	Concentration of weight		
	F1 (5%) per gram	F1 (7.5%) per gram	F1 (10%) per gram
Patchouli oil	12.5	18.75	25.00
Carbopol 940	1.25	1.25	1.25
Span 20	7.5	7.5	7.5
Tween 80	7.5	7.5	7.5
Propylene glycol	512.5	512.5	512.5
Methyl paraben	0.025	0.025	0.025
Propyl paraben	0.125	0.125	0.125
Alpha tocopherol	0.025	0.025	0.025
TEA	7.5	7.5	7.5
Glycerin	12.5	12.5	12.5
Aquadest	94.3 mL	91.2 mL	88.0 mL

Data Analysis

The data collection of the physical properties of the preparation was carried out by looking at the differences in each formulation using a One-way ANOVA test. While the irritation test was carried out by looking at the presence of erythema and edema score, which was calculated using the equation of primary irritation score (equation 1):

Equation 1. Primary irritation score:

$$\frac{\text{sum of edema and erythema grade all rabbits in group) / number of grading periods}}{\text{Number of rabbits in the group}} \quad (1)$$

The irritation test assessment involving a veterinarian is according to irritation guide at The National Food and Drug Department. The evaluation of skin reaction is presented in Table 2.

The results of observations are given a score of 0 to 4 according to the severity. The level of irritation is calculated based on the score of severity Table 3.

Table 2. Evaluation of Skin Reaction

Formation	Score
Erythema and eschar	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined	2
Moderate to severe erythema	3
Severe erythema to slight eschar formation	4
Total possible erythema score	4
Edema	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema	2
Moderate edema	3
Severe edema	4
Total possible edema score	4

Table 3. Response of category irritation on rabbit

Formation	Score
Negligible	0.0-0.4
Slight	0.5-1.9
Moderate	2.0-4.9
Severe	5.0-8.0

RESULT AND DISCUSSION

Preliminary testing is done by trial and error. In this study, spray gel samples were made using patchouli oil using a spray applicator with concentrate 5%, 7.5%, and 10%. The components used in this study were patchouli oil, carbopol 940, propylene glycol, triethanolamine (TEA), methyl paraben, propyl paraben, alpha tocopherol, tween 80, span 20, and distilled water. The use of carbopol 940 functions as a gelling agent. The choice of carbopol 940 as a base is because carbopol is hygroscopic, stable in acid and alkaline conditions, soluble in water, and has been widely used in the manufacture of semisolid samples.

Propylene glycol act as a humectant, solvent, and plasticizer. Triethanolamine serves as pH stabilizer, methyl paraben, and propyl paraben as preservatives with a maximum concentration of 0.3%, alpha tocopherol acts as an antioxidant, because the sample is in semisolid form, which is easily oxidized. The selection of tween 80 as a solubilizing agent was used to increase the solubility of essential oils in the emulgel sample and distilled water as a solvent. The use of surfactant is based on the suitability of the HLB value needed to form a stable and not easily broken emultion. Anggraeni et al., (2020) stated that the ability of an antimicrobial agent to eliminate the viability of microorganisms depends on the concentration of the antimicrobial agent. By the observations, it was found that the average diameter of the inhibition zone was getting bigger as the concentration of patchouli oil was added. The combination of tween 80 and span 20 is expected to produce gel preparation with characteristics good physique and can increase the activity patchouli oil antibacterial.

Research by (Swamy & Sinniah, 2015), with patchouli oil soap formulation, comparison results of the inhibition zone of *Staphylococcus aureus* ATCC 25923 between F0 (0.00%), F1 (0.05%), F2 (0.50%), and F3 (1.00%) showed that F3 with 1% patchouli oil concentration had the largest inhibition zone compared to F0 (negative control), F1 (0.05%), and F2 (0.5%). The liquid soap formula without patchouli oil (F0) also gave an inhibition zone of 11.29±2.60 mm which was higher than the soap base made in the study of Sarosa et al., (2018) which gave an inhibition zone diameter of 7.53 mm. This is due to the composition of liquid soap, which is formulated to contain additives such as propylene glycol, methyl paraben, and propyl paraben which also have antibacterial properties. The addition of propylene glycol a is able to provide a humectant function as well as an antimicrobial preservative. Methyl paraben and propyl paraben have broad spectrum antimicrobial activity over a wide pH range (Anindhita & Oktaviani, 2020). In F1, F2, and F3 the diameter of the inhibition zone was larger than F0 respectively, namely 14.60±2.45 mm, 15.51±0.44 mm, and 17.97±0.71 mm. It can be seen that the addition of patchouli oil can increase the diameter of the inhibition zone, which means that its antibacterial activity increases.

Patchouli oil is rich in sesquiterpene content, with the main compound being patchouli alcohol (patchoulol) (Swamy & Sinniah, 2015). Research by (Dai et al., 2012) reported that *Staphylococcus aureus* ATCC 25923 treated with patchouli oil would cause intra structural damage to its DNA, cell walls, and cytoplasmic membranes. The antibacterial mechanism of patchouli oil acts on the DNA of Staphylococcus aureus ATCC 25923 and causes abnormal cell division (Dai et al., 2012). The production of patchouli oil spray gel was carried out by modifying the concentration of the active substances, namely 5%, 7.5%, and 10%, respectively. The results of the spray gel were then tested for their physical properties, including organoleptic tests, pH, homogeneity, viscosity, dispersive adhesiveness, and spray power (Table 4).

Table 4. The results of the physical properties of patchouli oil spray gel

Physical properties test	Formulation		
	Formula 1 (5%)	**Formula 2 (7.5%)**	**Formula 3 (10%)**
Organoleptic test			
Color	yellowish white/bone white	yellowish white/bone white	yellowish white/bone white
Aroma	distinctive weak aroma of patchouli oil	distinctive weak aroma of patchouli oil	distinctive strong aroma of patchouli oil
Texture	Slightly liquid	Slightly thick	thick
Homogeneity	Homogeneous	Homogeneous	Homogeneous

The results of the third organoleptic test formulation of patchouli oil spray gel obtained a distinctive aroma of patchouli oil. The spray gel concentration of 5% and 7.5 % patchouli oil has a weak aroma, while the spray gel preparations with a concentration of 10% have a strong aroma. The results of the organoleptic test of the three spray gel formulations were carried out through sensing by looking at the color, aroma and texture of the preparation. The test results of the preparation can be seen in Table 4. The sample result producing yellowish white/bone white color with a distinctive aroma of patchouli oil. The texture obtained is in accordance with the spray gel, which is semisolid. The more concentration of patchouli oil is added, produces the same color. Meanwhile, an increase in spray gel concentration of 10% has a distinctive patchouli oil aroma. The homogeneity test was carried out to determine the mixing of the components of the ingredients in the preparation. The homogeneity test of the spray gel was carried out by smearing each spray gel on the object glass to observe its homogeneity. From the results of the homogeneity test, it was found that there were no lumps of particles on the object glass. This is in accordance with the requirements, which in preparation must show a homogeneous component of the material, and there are no lumps of particles. Spray gel is considered homogeneous if there are no coarse grains on the glass surface. The composition of the gel is said to be homogeneous if there is an even color equation and no different particles are found (Titaley et al., 2014). Gel spray formulation can be seen in Figure 1.

Figure 1. Spray gel formulation

The pH test is a degree of acidity test and is also part of the examination of chemical properties to predict the stability of the sample. The pH of the spray gel sample must be adjusted to the pH of the skin (Zulkarnain et al., 2013). This is intended to minimize irritation to the skin.

Change in pH values may occur due to the choice of Carbopol 940, which has an acidic pH of 3, that in the manufacturer of gel formulations with gelling agent Carbopol 940 it is necessary to add certain base neutralization. Neutralization will produce a repulsive force on the COO-carbopol group that the structure becomes more rigid, and the viscosity increases. TEA was chosen as a neutralizer because it has a pH of 10.5 and can help gelling agent (Ckarbopol 940) neutralizer. The test results using pH paper show the results with the average sample having a pH of 5.3. These results fall into the

range of requirements of the National Standards Agency, namely SNI 16-4380-1196 for human skin pH, which ranges 4.6-6.5.

Table 5. The result of pH, viscosity, dispersion, and adhesion of patchouli oil spray gel preparation

Formula	pH	Viscosity (dPa.S)	Dispersion (cm^2)	Adhesion (second)
F1	5.39 ± 0.30	136.67 ± 15,28	6.19 ± 0.67	1.66 ± 0.36
F2	5.45 ± 0.18	140.00 ± 17.32	5.96 ± 0.45	1.60 ± 0.38
F3	5.74 ± 0.23	143.33 ± 15.28	5.74 ± 0.47	1.67 ± 0.33

pH testing (Table 5) in a sample is very important because if the results are not in the standard pH range, it will have an adverse effect on skin health. If the pH is too acidic, it can cause the skin to shrivel and break, while a pH that is too alkaline can cause the skin to become dry, scaly, and cracked. This skin condition may cause discomforts, such as itching and burning, during and after the use of the spray gel (Sharon et al., 2013).

The viscosity test on the spray gel sample aims to determine whether the sample is easily delivered through the spray applicator used or not. Viscosity is one of the quality parameters of topical preparations, which is a preparation resistance to flow. Viscosity is responsible for the physical properties of a gel preparation and plays an important role in increasing gel stability. Based on the gel viscosity test using a viscometer, the viscosity results obtained were analyzed using the One Way Anova parametric test and showed that there was a significant effect (0.00 < 0.05) between the addition of patchouli oil concentration on the viscosity.

The higher concentration of patchouli oil added, the higher the viscosity of the spray gel preparation. Viscosity test greatly affects the results of other tests, such as adhesion test, dispersion test, and spray pattern test. The viscosity of the spray gel sample is made lower so that it is easily delivered through the spray applicator to reduce contamination or contact when applying the spray gel to the skin. In addition to the selection of a gelling agent, carbopol 940 has the ability to affect high stability, is resistant to microbes, and has been widely used in the pharmaceutical and cosmetic world. The efficiency of carbopol 940 is great so that at low levels, it can provide a significant viscosity response (Allen & Loyd, 2002).

Adhesion test was conducted to determine to which extent the spray gel's ability to adhere to the skin so that the active substance can retain to increase its effectiveness. The test of the adhesiveness of the spray gel sample is performed to determine the adhesive ability of a sample after being sprayed. This test was conducted by measuring the adhesive time of the spray gel using a slide and a stickiness tester. The calculated time is then used as a test value for adhesion (Wasitaatmadja, 1997). In this study, it was found that the higher the concentration of patchouli oil, the higher the adhesion of the preparation. The results of the adhesion test for patchouli oil spray gel preparations with concentrations of 5%, 7.5%, and 10% had good adhesion, which was more than 1 second. The increase in viscosity will increase the adhesion of the formulation. This is in accordance with the requirements for adhesion to semisolid preparations preferably more than 1 second. The third formula for spray gel preparations, 10%, has the longest adhesion. This shows that the addition of patchouli oil can increase the adhesion of spray gel preparations. The combination of carbopol 940 as a gelling agent was able to increase the adhesion of the preparation. Increasing the concentration of spray gel preparations affects the longer the stickiness of a preparation. This adhesion test shows the ability of the preparation to adhere to the site of application. The longer the preparation can be attached, the longer the active substance can be in contact with the application site, so it is expected that its antibacterial effect can be more optimal (Ismarani et al., 2014).

The dispersion test was performed by weighing 0.5 grams of gel and then placing it on a glass with a diameter of 15 cm. The dispersion test was performed to determine the ability of the patchouli oil spray gel sample to spread on the skin. The test was carried out for 1 minute by measuring the diameter of the gel spread. The dispersion of the gel can also be calculated using the formula S = m*l/t (Haneefa et al., 2010). The dispersion test at each concentration aims to determine the ability of the patchouli oil spray gel sample to spread on the skin. The ointment sample, which is convenient to use, has a spread of 5 to 7 cm. A sample will be preferred if it can spread well, making it easier to use and more comfortable. From the results of this study, it was found that the spray gel had a dispersion that met the dispersion parameters that were comfortable for the skin. The characteristic of patchouli oil which is slightly viscous, affects the viscosity of the spray gel sample. Spray gel F1, F2, and F3 varying results. The variation in spraying patterns formed from the spray gel preparation is influenced by the spraying distance and the viscosity of the preparation. In the study, the spraying distance was directly proportional to the diameter of the spray pattern. The greater spraying distance, the greater the spraying pattern produced. The spraying pattern in F1 and F2 tends to produce an elongated and spreading pattern. While the pattern. Spraying in F3 tends not to spread and is only at one straight point from the spray, small in shape with an average diameter of 1-2 cm. This is because in F3 the preparation is slightly thicker than F1 and F2 (Table 6).

Table 6. Weight of the droplet (g) formulation per spray

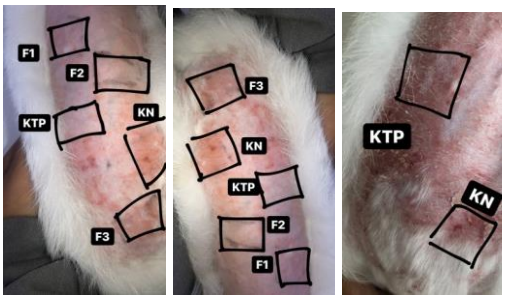
Formulas	Average weight/spray± SD (g)
1 (5%)	0.11 ± 0,00
2 (7.5%)	0.11 ± 0.01
3 (10%)	0.11 ± 0.00

The results of the inspection of the delivery weight of the preparation for each spray showed that there was no significant difference between each formula. This shows the effectiveness of the applicator used in delivering reproducible amounts of the gel dosage formula per spray.

The target of the viscosity is chosen *in range* because if the value of the viscosity is getting higher, it will cause the dispersion to decrease. For the dispersion, the goal maximize was chosen because the average dispersion obtained from the 3 formulas was only around 5.96 cm^2, even though the greater the dispersion of the semisolid (gel) preparation, the better the preparation. The purpose of the adhesion was chosen *in range* because the data obtained from the 3 formulas met the requirements (more than 1 second), there were no special requirements regarding the adhesion of semisolid preparations, but should have adhesion of more than 1 second (Zats & Gregory, 1996).

The purpose of the acute dermal irritation test is to determine whether there is an irritating effect on the skin and to assess the characteristics of a substance when exposed to the skin. The results of the observations are stated in the irritation test score assessment in each group. The irritation test was examined by degree of erythema (redness reaction) and edema (swelling reaction) that appeared on rabbit (*Oryctolagus cuniculus*) skin. The test was then observed in vivo on rabbit (*Oryctolagus cuniculus*) at 0, 24, 48, and 72 hours after application of spray gel preparation. Irritation test assessment involving a veterinarian based on irritation guide at National Food and Drug Departement. Alteration were scored according to the criteria shown in Figure 2 and Figure 3, and eact test material was judged overall in skin irritation classification on rabbit using criteria listed.

The test is carried out by taking care of the ethics of the client at the ethics committee of Sultan Agung University with a number 305/IX/2021/ Ethics Committee. The rabbit skin was shaved and given the treatment formulation. The irritation test was observed by the presence or absence of erythema and edema. The test results showed very mild irritation, as indicated by the primary irritation index score of 0.0. No erythema and edema were found during 72 hours of observation. Patchouli oil spray gel samples with concentrations of 5%, 7.5%, and 10% did not show any erythema and edema effect.



(a) (b) (c)

Figure 2. irritation test result in rabbits in various treatment group at (a) 24, (b) 48, and (c) 78

CONCLUSION

The results showed that the increase in concentration affected the physical characteristics of the preparation. The higher the concentration causes the aroma of patchouli oil to become more concentrated and thicker, and the spreadability gets smaller. Formula 3 has the longest adhesion among other formulas. Meanwhile, in terms of color, F3 has a darker color than F1 and F2. The results of the examination of the delivery weight of the preparation for each spray showed that there was no significant difference between each formula. The average pH of the three formulations was 5.3, in accordance with the pH requirements of the skin. From the three formulations, the second formulation with patchouli oil concentration of 7.5% showed an optimum result as a spray gel sample, where the consistency was not too thick, the adhesive spread was sufficient, and the pH range was within the requirements. But from the three formulation the pH was stated to be in the required range. Meanwhile, to support the acceptability of each formulation, it is necessary to carry out hedonic test. The results of the irritation test showed that the three formulations have a very mild irritation effect.

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