Formulation and the release of eugenol from cream using glycerin base

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Abstract. Eugenol consist of phenol, which have antibacterial effect. The aims of this research were to observed the characteristics of cream dosage form, eugenol released profile, and determined the effect of glycerin as water base through in vitro evaluation. Cream was made with glycerin as enhancer and water base. Various concentration of glycerin (10%, 15%, and 20%) were added to eugenol cream. Cream evaluation included organoleptic, homogenity, pH, stickiness, spreadability, viscosity, and eugenol released test from cream base of each formula. Drug released test was carried out eith Erweka Dissolution tester using apparatus 5 paddle orderisk. Phosphate buffer 7.4±0.05 was using as medium, with 32˚C and 200 rpm rotation. The resul showed that eugenol can be formulated into cream, because of the evaluation has met the requirments of test quality. Those quality were organoleptic, homogenitu, pH, stickiness, viscosity and spreading test. Concentration analysis of eugenol cream in glicerin various concentration were evaluated using UV-Vis spectrophotometer. The result showed that total concentration of eugenol in cream were 107.11%; 99.91%; 105.63%. The result of eugenol released test were 11.53%; 11.38%; 10.96%. Eugenol cream with 10% of glycerin concentration has the highest perfomance as enhancer and water base.

1. Introduction
Indonesia is one of the largest producer of essential oil in the world. It can produce 50-150 types of essential oils available [16]. Essential oil has been proven that it gave benefits for health. One of the type of essential oil is eugenol. It were stated that eugenol has anti-inflammatory activity for acute lung injury (ALI) and antioxidant activity [17]. According to other research, it was revealed that eugenol has anticancer activity, antidepressant activity and anthelmintic activity [1]. Due to eugenol activities that have been proven before, it was important foe eugenol to be developed as a dosage form. In order to give more benefits of eugenol for health.

One way to utilize eugenol potential is by developin it into a high quality of pharmaceutical product, whic can be used widely by society. The requirement of pharmaceutical product to qualified are safe to use, effective for medicine, comfortable for patient, and having both physical and chemical stability. It can guarantee the quality of production and distribution process until it can be consumed by patient. Pharmaceutical preparation has big potential to developed such as topical products, one of them is cream. Topical product is use on skin surface to give it local effect. It has been proven that eugenol have pharmacologic activity which could be developed as topical product for antibacterial, anti-inflammatory, anthelmintic, antioxidant, and anticancer. Morover, eugenol has antidepressan ctyivity which became an additional value.
The cream preparation, excipient or base is often to use as additional material usually to repair its consistency. It also can be used as cosolvent by increasing the solubility of the drug ingredients. Increasing solubility of cream will make drug easy to release from their base and influence the activity [5]. A base is suitable for cream if it were not strongly bind with other ingredients, since the drug must release immediately from base before it penetrated to skin [9]. There are two steps for topical drug before it gives effect. Firstly the drug must be able to released from their base and go to skin surface. Secondly they can penetrate through the skin membrane to achieve its action[6]. This research aims to find out the correlation between physical stability and releasing profile of cream eugenol with various concentration of glycerin (10%, 15%, and 20%). These amount of optimal glycerin will increase eugenol penetration into the skin, so the optimal therapy effect can be reached.

2. Experimental

2.1 Materials
Eugenol, Glycerin (Bratako), Sodium Lauril Sulphate (Bratako), Vaseline Album (Bratako), Stearic Acid (Bratako), Setil Alcohol (Bratako), KH2PO4 (Merck), NaOH (Merck), Ethanol p.a (Merck), cellophane membrane (Merck), Dissolution tester, Viscometer (Rion vt-04E Co, TLD) , pH meter (Lutron), Spektrohotometer UV-Vis (Genesys ™).

2.2 Methods
2.2.1. Cream Formulation
Cream was made by melting oil base including cetyl alcohol, vaselin album, stearic acid above the waterbath. Then heat the water base and dissolving glycerin, sodium lauryl sulphate, and aquadest. The melting oil base was put into a warmer mortar, then eugenol was added. After that the oil phase was mixture with water base, and stirred until cream emulsion was formed.

| Material                | Amount (%) | F1 | F2 | F3 |
|-------------------------|------------|----|----|----|
| Eugenol                 | 1.25       | 1.25| 1.25|
| Glycerin                | 10         | 15 | 20 |
| Sodium lauril sulphate  | 2          | 2  | 2  |
| Vaseline Album          | 25         | 20 | 15 |
| Stearic acid            | 10         | 10 | 10 |
| Cetyl Alcohol           | 3          | 3  | 3  |
| Aquadest                | Ad 100     | Ad 100| Ad 100|

2.3. pH Test
pH evaluation was determined by using pH meter (Lutron). Cream was taken at a certain amount and it was mixed with aquadest. A mixture of cream and aquadest was analysed using pH meter. pH meter was placed in the mixture. The change on the scale of pH meter can be seen. The number displayed on pH meter was an exact pH from the product [14].

2.4. Stickiness Test
0.25 gram of sample was put on two glass objects. Then pressed the glass with 1 kg load or above, and left it for 5 minutes. After that, the glass objects were place on the tool that was given load 80 gram. The time was recorded until the two glass objects were separated [11].

2.5. Spreadability Test
0.5 gram of cream was placed on the midle of circular glass, and left it for 1 minute. Distribution diameter of cream was measured. After that it was added with 150 gram additional load and left it for 1 minute, then constant diameter of cream was measured.
2.6. Viscosity Test
The viscosity of cream was measured by using viscosimeter Rion vt-04E Co, TLD. The bowl was filled with a half of the cream sample to be tested. The rotor was put in the middle of the bowl containing the cream, it was turned on to start rotating, the viscosity pointer will automatically move to the right. Once it is stable, it is displayed on the scale on the viscometer.

2.7. Determining Composition
1 gram of eugenol cream was diluted into 10 mL of ethanol, and then stirred for 15 minutes in 25 °C. Cream solution was distilled, and filtrate was generated. After then, it was diluted and analyzed using UV-vis spectrophotometer at a wavelength of 200-400 nm.

2.8. Eugenol Release from Base
Release test uses type 5 of dissolution test tool. Membrane used was cut and adjusted to the area of diffusion disc. Chamber was filled with 500 mL of phosphate buffer and the temperature was set at 32 ± 0.5 °C. Rotational speed was adjusted into 200 rpm. Medium fluid was taken as much as 10 mL at 0, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420, and 480 minutes. The sample liquid was analyzed using UV-vis spectrophotometer at wavelength of 283 nm[13].

3. Results and Discussion

3.1 pH Test
Potential for Hydrogen or pH test is aim to find out the safety and comfort of preparations when it used on skin. pH profile can determine the stability of an active ingredient when it is conditioned in an acidic or alkaline atmosphere[10]. According to SNI-16-4399-1996, the ideal pH should be in line with the skin pH; if the pH is too acidic, it will cause irritation to the skin. Meanwhile, if the pH is too alkaline, it can result in scaly skin[12]. Based on table 2, it is seen that the three formulas have been met the requirements of SNI.

3.2. Stickiness Test
The test aim to find out how long cream is able to attach on skin surface. The test conducted for 4 weeks shows that formula 1 has the longest stickiness, while the formula 3 has the fastest stickiness. It is because formula 1 has the highest oil-based concentration (Vaseline album) than other formula. Formula 3 has the lowest oil based concentration so it make formula 3 has the fastest time for cream to stay on skin surface.

3.3. Spread Test
Distribution test aims to find out cream ability to spread evenly while it is applied to the skin. A good cream is expected to spread easily on the skin surface without significant pressure. The easier preparation spread on the skin, the biggest the skin contact to the medicine will be. Becuase of that the absorption of the medicine will be more optimal. Good spread for semisolid preparation is 5-7 cm[15]. Data of spread test can be seen in table 4.

3.4. Viscosity Test
The result of viscosity test can be seen on table 5. Formula 1 has the highest viscosity while formula 3 has the lowest viscosity. It is due to highest concentration of glycerin will decrease cream viscosity. Decreasing viscosity can be caused by the increasing of globule. Thinner or softer cream will easy to move in dispersion medium and the globule will form a bigger particle. Biger size of globule will cause the reduction on the area of cream surface. Good cream viscosity according to SNI 16-4399-1996 is 2,000-50,000 cP or equivalent to 20-500 d.Pas. Only formula 1 with a base of 10% glycerin has viscosity according to SNI.
3.5. Determining Composition
Test for determining composition was presented in ratio between measured composition and theoretical composition. The result of eugenol composition can be seen in table 6. It showed that all formulas have met the requirements, which is in ratio of 80-11%[3].

3.6. Eugenol Release Test
The percentage of released eugenol can be seen in table 7. The value showed that eugenol composition in the buffer medium. Besides accumulated amount, diffused eugenol is left in the cellophane membrane. It can be concluded that the total amount of eugenol was able to diffused, has higher value than measured value in medium liquid. The most important factor for active substance released were preparation formulation, testing medium, membrane type, intensity of movement and temperature, selected excipient (Vaseline and glycerin), and viscosity of preparation which will influence the active substance release[15]. Eugenol has a phenol component so eugenol is easily soluble in glycerin. A active substance that is soluble in polar solvents in large quantities causes the drug to be difficult to release and penetrate through the membrane. For glycerin it is as a humectant. Humectants work by forming a layer on the surface of the skin to protect. Membranes coated with glycerin make eugenol difficult to penetrate and diffuse. Therefore eugenol can be released from the formula with the highest concentration of glycerin.

| Table 2. pH test of eugenol cream |
|----------------------------------|
| pH | Week 1 | Week 2 | Week 3 | Week 4 |
|----|--------|--------|--------|--------|
| Formula 1 | 6.50 | 6.53 | 6.53 | 5.80 |
| Formula 2 | 6.50 | 6.50 | 6.50 | 6.60 |
| Formula 3 | 6.60 | 6.60 | 6.60 | 6.60 |

| Table 3. Stickiness test of eugenol cream |
|------------------------------------------|
| Stickiness Test | Week 1 | Week 2 | Week 3 | Week 4 |
|-----------------|--------|--------|--------|--------|
| Formula 1 | 2.67 | 4.34 | 3.33 | 3.58 |
| Formula 2 | 1.56 | 3.85 | 2.25 | 2.56 |
| Formula 3 | 1.51 | 3.61 | 2.41 | 2.51 |

| Table 4. Spread test of eugenol cream |
|--------------------------------------|
| Spread Test (cm) | Week 1 | Week 2 | Week 3 | Week 4 |
|------------------|--------|--------|--------|--------|
| Formula 1 | 4.50 | 4.23 | 4.67 | 5.40 |
| Formula 2 | 6.40 | 5.52 | 6.40 | 6.22 |
| Formula 3 | 6.83 | 5.90 | 6.60 | 6.40 |

| Table 5. Viscosity test of eugenol cream |
|----------------------------------------|
| Viscosity(dPas) | Week 1 | Week 2 | Week 3 | Week 4 |
|-----------------|--------|--------|--------|--------|
| Formula 1 | 80 | 80 | 80 | 80 |
| Formula 2 | 40 | 35 | 35 | 35 |
| Formula 3 | 30 | 30 | 25 | 25 |
Table 6. Determining composition of eugenol

| Formula | Replication | Sample weight (mg) | Eugenol theoretical concentration (ppm) | Research eugenol concentration | Average % recovery |
|---------|-------------|--------------------|----------------------------------------|-------------------------------|-------------------|
|         | 1           |                    |                                        |                               |                   |
| 1       | 1           | 1000               | 1250                                   | 1338.75                       | 107.11±0.37       |
|         | 2           | 1000               | 1250                                   | 1342.76                       | 99.91±2.93        |
|         | 3           | 1000               | 1250                                   | 1333.88                       | 105.63±3.80       |
| 2       | 2           | 1000               | 1250                                   | 1267.75                       |                   |
| 3       | 2           | 1000               | 1250                                   | 1272.63                       |                   |
|         | 3           | 1000               | 1250                                   | 1270.85                       |                   |
| 3       | 2           | 1000               | 1250                                   | 1367.11                       |                   |
|         | 3           | 1000               | 1250                                   | 1322.95                       |                   |

Table 7. Amount of eugenol that penetrated through in vitro test (release profile)

| Formula | % eugenol penetrated | SD | CV (%) |
|---------|----------------------|----|--------|
| 1       | 11.290               | 0.298 | 2.639    |
| 2       | 11.773               | 0.299 | 2.541    |
| 3       | 9.503                | 0.256 | 2.695    |

4. Conclusion
The difference in base concentration will affect the stability of the cream such as viscosity, spreadability, and eugenol release profile. The higher the concentration of glycerin, the smaller the viscosity, the wider the spread of power, and eugenol is difficult to release.

Acknowledgment
Thank you to Sebelas Maret University for the financial support.

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