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INVITRO OF FORMULATION AND EUGENOL RELEASE TEST USING VASELINE ALBUM BASE IN OINTMENT PREPARATION

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eugenol, drug release, ointmnent, vaselinealbum

Abstract

Eugenol is an antibacterial agent that is able to inhibit or be bactericidal in Escherichia coli, Pseudomonas, Salmonella typhi and Staphylococcus aureus bacteria. The aimed of this research was to observe the characteristics of dosage form and release of eugenol ointment, and also to determine the effect of vaseline album concentration as a base and penetration enhancer of eugenol in the ointment preparation through membrane in vitro. Ointment was maked with vaseline album as base of ointmnet. Variation concentration of glyserin (82 g, 8 g, and 78 g) was added to eugenolointmnet. The evaluation included organoleptic, homogeneity, pH, viscosity, diffusion measurement, adhesion power, the contents of eugenol were analyzed by using UV-Vis spectrophotometry, and also eugenol release test from ointment base of each formula. Drug release test was carried out with Erweka Dissolution Tester with apparatus 5 paddle overdisk in phosphate buffer 7.4 ± 0.05, temperature 32°C, 200 rpm. The results showed that the differences in base vaseline albumin concentration influence ointment stability such as adhesion, dispersion, and eugenol dissolution profile. The increasing the concentration of vaseline album base, followed also by the increasing amount of eugenol released.

Introduction

Eugenol compounds are widely researched and developed because of the very diverse properties. The biological activities of eugenol include anti-fungal, antibacterial, anti-carcinogenic, hypo-allergenic, antioxidant, antimutagenic, and anti-insect properties(Daniel, et al., 2009). Eugenol can be obtained through the isolation of natural materials, synthesis and biotechnology methods, which involve several microorganisms such as Pseudomonas sp, Escherichia coli, and Bacillus cereus (Mishra & Sachan, 2013). Besides being used in the medical field, eugenol is also widely used in industrial fields such as the food industry, the perfume industry, the agricultural industry, the textile industry, and others.

Eugenol is an antibacterial agent that is able to inhibit or be bactericidal in Escherichia coli, Pseudomonas, Salmonella typhi and Staphylococcus aureus bacteria(Merchese, et al., 2017). Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) eugenol in Staphylococcus aureus bacteria is at a concentration of 625 µg / mL (Pilleti, et al., 2017). Based on research, it was stated that the concentration of eugenol as an antibacterial Listeria monocytogenes and Salmonella enteritidis in nanoemulsion preparations was 1.25%(Hu, et al., 2016).

Utilization of eugenol so that more leverage can be formulated into a preparation that is able to increase the comfort of use of eugenol. As an alternative to antibacterial treatment, eugenol is made or formulated in the form of pharmaceutical preparations aimed at making it easy and practical in its use. One form of pharmaceutical preparation that can be made is topical preparations, for example ointments.

One form of maintaining ointment stability is in selecting suitable ointment bases. One of the most commonly used base ointments is vaseline album. Ointment is a pharmaceutical preparation in the form of semi-solid or semisolid, and is used on the surface of the body or skin.

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Release test of the active ingredient eugenol from an ointment base is intended to find out that the preparations made have been optimum. Several factors that need to be considered when penetrating drugs through a membrane include the type of base, solubility of the active substance in the base, and the pH of the base. Testing the release of active substances from carriers in vitro is a more efficient method in characterizing the absorption and penetration of drugs through the skin membrane (Prabawati, 2015).

Based on this background, the active ingredient formulation of eugenol is done in the ointment preparation, and determine which base is the most effective for eugenol ointment. The physical quality of the ointment is inseparable from the selection of a suitable base. The base functions as a carrier, protector and skin softener, must be able to release the drug optimally (must not damage or inhibit the action of therapy), and may be suitable for certain diseases and certain skin conditions (Sulaiman & Kuswahyuning, 2008). Through in vitro testing it will be known the ability of ointments to release active substances from the base.

Materials and method

Tools
UV-Vis spectrophotometer (Thermo Scientific Genesys 10S), Dissolution Test Equipment (RC-6), pH Meter (Lutron PH-208), Viscometer (Rion).

Materials
Eugenol, KH2PO4, ethanol, aquades, cellophane membranes.

Eugenol Ointment Formula

Table 1. Eugenol Ointment Formulation

Materials	Formula 1 (gram)	Formula 2 (gram)	Formula 3 (gram)
Eugenol	4	4	4
Vaselin album	82	80	78
Glyserin	7	9	11
Stearyl alcohol	3	3	3
Cholesteroly	3	3	3

How to make Eugenol Ointment: Vaseline album is heated on a waterbath until it melts. After chilling, mixed with stearil alcohol, cholesterol, stirring until homogeneous. Add glycerin, and eugenol, stir until homogeneous.

Organoleptic Test
The preparation is observed with the five senses to describe the shape, homogeneity, color, and odor. The test is carried out before and during the storage period, with an interval of one week for one month.

Homogeneity Test
Ointment preparations taken 0.5 grams, placed on glass preparations, then flattened, and observed visually (Hernani, et al., 2012). The test is carried out before and during the storage period, with an interval of one week for one month.

PH Test
PH testing using a pH meter, by entering 1 gram of ointment, dissolved in 10 mL of distilled water, then allowed to stand for a while, and measured the pH value (Paju, et al., 2013). The test is carried out before and during the storage period, with an interval of one week for one month.

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Adhesion Test
A total of 0.25 grams of ointment is placed on a glass preparation, then covered with other glass preparations on it, and pressed with a weight of 1 kg, and left for 5 minutes. After 5 minutes, the prismatic glass was pulled with a weight of 80 grams until the two preparatory glass each released (Nurlaela, et al., 2012). The test is carried out before and during the storage period, with an interval of one week for one month.

Scattering Test
A 500-mg ointment was weighed, and placed in the center of a petri dish that had been given a millimeter scale. Petri dishes are stacked with other glass, wait for 1 minute, then the distribution diameter is measured. Then 100 grams of weight were added to the glass lid, waited for 1 minute, and measured in diameter. Testing is done with the addition of each load of 50 grams, each additional load is waited for 1 minute(Nurlaela, et al., 2012). The test is carried out before and during the storage period, with an interval of one week for one month.

Determination of the maximum wavelength of eugenol
The maximum wavelength of eugenol is determined using a UV-Vis spectrophotometer at λ 200 - 400 nm; phosphate buffer solution is used as a blank. The wavelength that produces the highest absorbance spectra is determined as the maximum wavelength of eugenol.

Determination of Eugenol Level in Ointments

Basic standard curve
The eugenol solution is made as much as 1250 ppm using ethanol solvent. As much as 1-gram base ointment mixed with eugenol solution, then added to 10 ml of ethanol. Base solution produced, filtered and diluted into several concentrations.

Determination of Content
A total of 1-gram ointment was dissolved in 10 ml of ethanol, then sterilized for 15 minutes at 25°C. Then filtered and diluted, and analyzed by UV-Vis spectrophotometer at a wavelength of 200-400 nm.

Release of Eugenol from Base
The eugenol release test from the base uses a Type 5 dissolution modification device. The size of the membrane used is adjusted to the area of the diffusion disk. The dissolution medium used is phosphate buffer as much as 500 mL, with a temperature of 32 ± 0.5°C, and a rotational speed of 200 rpm. At 0, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420, and 480 minutes, 10 mL of dissolution medium was taken. The sample fluid is taken at any time, then analyzed using a UV-Vis spectrophotometer at a wavelength of 283 nm (Qisti, 2017).

Result and discussion

Organoleptic Ointment.
The results of making ointments, with variations in the concentration of vaseline and glycerin base, provide three ointment formulations with a light brown color, the characteristic odor of eugenol, and have the same consistency for four weeks of storage. This means that the difference in base concentration variation does not affect the organoleptic cream for four weeks of storage.

Table 2. Organoleptic Test Results and Homogeneity of Ointments

Formulation	Assessment		
	Color	Odor	Consistency
Formula 1	Light Brown	TypicalEugenol	Soft, semisolid, homogeneous

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Formula 2	Light Brown	TypicalEugenol	Soft, semisolid, homogeneous
Formula 3	Light Brown	TypicalEugenol	Soft, semisolid, homogeneous

Homogeneity Ointment

Ointment can be said to be homogeneous if there is no visible separation between its constituent components. Homogeneity shows the mixing of ingredients in formulas (Rieger, 1994). Homogeneity test results are in accordance with the requirements of Pharmacopoeia of Indonesia, which is when the preparation is applied to a piece of glass or suitable transparent material, showing a homogeneous arrangement that can be seen, in the absence of clusters of particles, and spread evenly(Depkes, 1979). The test results showed that there was no change in the ointment, and the ointment remained homogeneous for four weeks of observation, this result showed that the organoleptic ointment had good stability. Thus, differences in the base concentration of each formulation did not affect the homogeneity of the ointment.

Ointment pH

This test was conducted to determine the acidity level of ointment preparations. And this test is intended to determine the nature of the ointment in irritating the skin. Normal skin ranges from 4.5 to 6.5. PH values that exceed 7 are feared to cause skin irritation (Rohmani & Muhammad, 2019).

Table 3. pHOinment Test Result

	Formula I	Formula II	Formula III
Week1	5,33	5,37	5,38
Week 2	5,35	5,37	5,37
Week 3	5,35	5,38	5,39
Week 4	5,36	5,36	5,38

The observation and measurement of ointment pH for four weeks did not experience a significant change. That the value of the data is still in the pH range of the ointment, which is not less than 4.5, and not more than 7. It can be concluded that the pH values are safe to use (Rohmani & Muhammad, 2019).

Ointment Adhesion

This test is carried out to see how long the *pandan* leaf extract ointment can stick to the surface of the skin, so that the active substance in the ointment is absorbed (Ansel, 1989). The longer the ointment sticks to the skin, the greater the effect.

Table 4. Oinment Adhesion Test Result

	Formula I (s)	Formula II (s)	Formula III (s)
Week 1	1,99	1,86	1,77
Week 2	2,11	1,92	1,77
Week 3	2,04	1,85	1,81
Week 4	1,97	1,94	1,88

The results of the adhesion test in Formula I had the longest time compared to other formulas.

Formula I, with the highest amount of vaseline base album, makes the ointment the longest adhesion, and allows the drug to disappear longer after being applied. The smaller the concentration of vaseline album base, then the adhesive power of the ointment will also be faster.

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Ointment Dispersion

The dispersion test for each preparation with a variety of base concentrations was carried out to see the ability to spread on the skin, where an ointment base should have a good dispersion to ensure satisfactory administration of medicinal ingredients. The difference in the dispersion is very influential on the speed of diffusion of the active substance across the membrane. The wider the membrane to which the preparation is spread, the greater the diffusion coefficient which results in drug diffusion increasing; so the greater the dispersion of a preparation, the better (Hasyim, et al., 2012).

Table 5. Oinment Dispersion Test Result

	Formula I (cm)	Formula II (cm)	Formula III (cm)
Week 1	3,67	3,56	3,48
Week 2	3,55	3,42	3,35
Week 3	3,57	3,43	3,33
Week 4	3,62	3,57	3,47

The dispersion test results in Formula I have the highest dispersion value and the widest diameter compared to other formulas, meaning that the ointment in Formula I is the best ointment because it is easily spread, and hopefully, the absorption of the drug will be more optimal.

Determination of Eugenol Maximum Wavelength

Determination of the maximum wavelength aims to determine the ability of UV-Vis spectrophotometer in detecting absorption of eugenol. The absorption results showed that the maximum wavelength of eugenol at 283 nm was in accordance with the literature (Indalkaar & Alookar, 2015).

Standard Curve

In making the standard curve, measured at the maximum wavelength at 283 nm, then a linear regression equation is made to determine the value of the linear correlation. The linearity parameter used in making the calibration curve is the coefficient of determination (R^2). From the eugenol calibration curve, the coefficient of determination (R^2) is close to 1. Linearity is recognized if the coefficient of determination (R^2) is more than 0.997 (Chan, et al., 2004), so the results show a linear relationship between concentrations with absorbance. The standard curve drawing for each formula can be seen in Figure 1, Figure 2, and Figure 3.

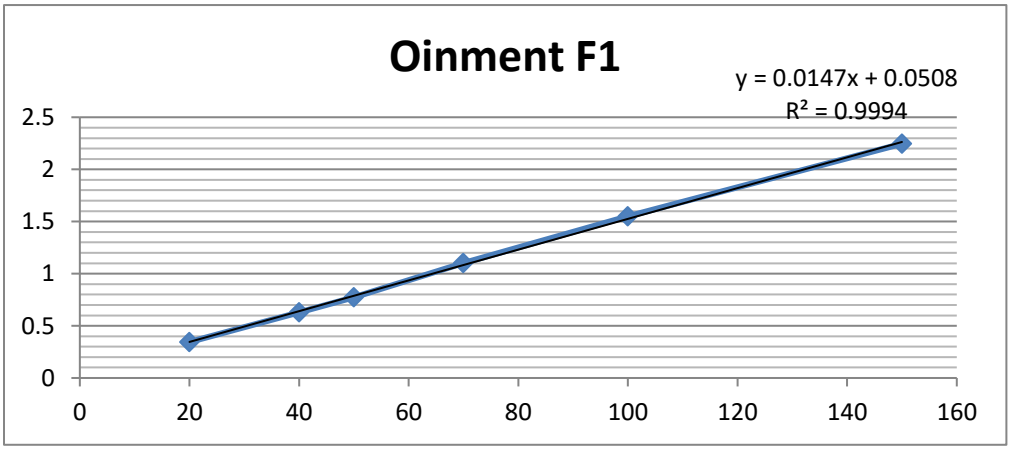

Figure 1. Formula I Basic Curve

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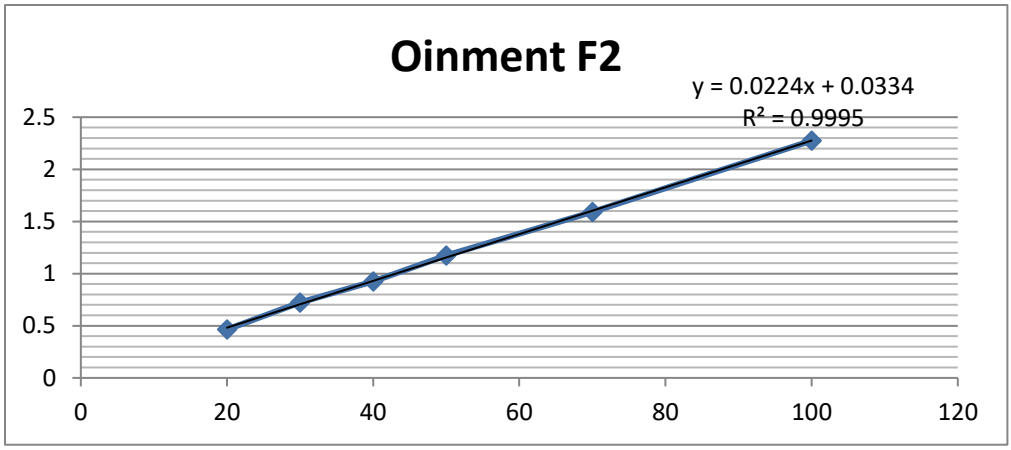

Figure 2. Formula I Basic Curve

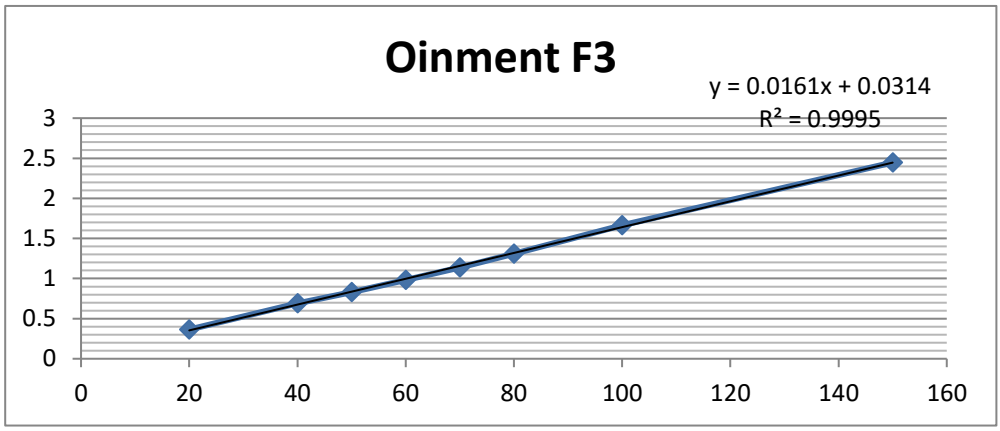

Figure 3. Formula I Basic Curve

Determination of Content

This Determination of Content aims to ensure the quality and safety of a product. The Determination of Content test is expressed as a percent ratio between the measured level result and the theoretical level. The results of the measurement of eugenol content show that all formulas are in accordance with the conditions determined according to the Association of Official Analytical Chemist which is in the ratio between 80 - 110%(AOAC, 1998).

Table 6. Measurement Results of Eugenol Contents in Formula I Ointment

Replication	Absorbance			Average Absorbance	% Content	Average % Content	SD	%CV
1	0,849	0,846	0,848	0,8476	108,41	105,47	4,2442	4,02
2	0,794	0,791	0,786	0,7903	100,61			
3	0,840	0,843	0,838	0,8403	107,41			

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Table 7. Measurement Results of Eugenol Contents in Formula II Ointment

Replication	Absorbance			Average Absorbance	% Content	Average % Content	SD	%CV
1	1,203	1,208	1,214	1,2084	104,91	96,99	7,2950	7,5
2	1,100	1,102	1,108	1,1034	95,54			
3	1,049	1,046	1,047	1,0474	90,54			

Table 8. Measurement Results of Eugenol Contents in Formula III Ointment

Replication	Absorbance			Averae Absorbance	% Content	Average % Content	SD	%CV
1	0,812	0,822	0,819	0,8176	97,66	95,09	2,6759	2,81
2	0,774	0,774	0,776	0,7746	92,32			
3	0,798	0,797	0,801	0,7986	95,30			

Eugenol Dissolution

Dissolution test aims to determine the amount of drug that is released from the medium to the dissolution medium. Semisolid preparations can have an effect if the drug ingredients can be separated from the base. Table 9 shows the amount of eugenol contained in the dissolution medium. The results showed that the greater the concentration of vaseline base album, the amount of eugenol released was also more numerous. Vaseline album including hydrocarbon ointment base type. The hydrocarbon base is softening the skin layer (emollient) because it is occlusive (leaving a layer on the surface of the skin) so that it will increase skin hydratation by inhibiting the evaporation of water in the skin layer. Due to hydratation of the skin layer, it might also increase drug activity. And the results of the study showed that the activity of eugenol release was increased due to hydratation of the skin layer by vaseline album.

Table 9. Release of Eugenol

Formula	Replication	% Eugenol Released	AverageEugenolReleased (%)	SD	CV (%)
I	1	9,676	9,265	0,358	3,864
	2	9,022			
	3	9,096			
II	1	8,022	7,867	0,187	2,397
	2	7,682			
	3	7,718			
III	1	7,367	7,399	0,104	1,399
	2	7,518			
	3	7,332			

Conclusion

a. Differences in base vaseline albumin concentration influence ointment stability such as adhesion, dispersion, and eugenol dissolution profile.
b. The increasing the concentration of vaseline album base, followed also by the increasing amount of eugenol released.

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