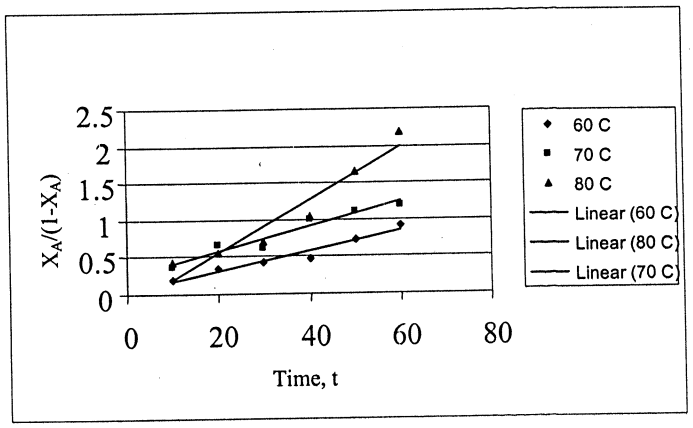

Figure 3. Correlation chart $X_A/((1-X_A)$ versus time

The most appropriate reaction order for this alcoholysis process is determined by choosing the smallest error value obtained from assessment of those three models. Model 1 has the smallest average error value, i.e.: 6,54 % and it can then be concluded that alcoholysis reaction of coconut oil is following first order to oil.

To determine reaction rate constant, Arrhenius equation is applied by using k data from Table 2. Equation obtained is $k = 0{,}0902\ e^{(-144{,}1220/T)}$ with error ....%.

Conclusion

1. Alcoholysis of coconut oil with sulphuric acid catalyst follows 1 order reaction to oil.
2. Correlation between k and T is according to equation $k = 0{,}0902\ e^{(-144{,}1220/T)}$ that apply for temperature range 60 - 80 °C

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ANTI-FUNGUS ACTIVITY TEST AND THE PHYSICAL CHARACTERISTIC TESTS OF VOLATILE OIL OINTMENT OF TEMU KUNCI RHIZOMA (*Boesenbergia pandurata* (Roxb) *Schlecht*) ON *Candida albicans* IN *VITRO.*

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ABSTRACT

Temu kunci rhizoma is one traditional medicine, which is frequently used by most people. Temu kunci rhizoma contains much volatile oil, which is useful for anti-fungus. The objective of research was to understand activity of anti-fungus and physical characteristic of volatile oil ointment of temu kunci rhizoma on *Candida albicans* in *vitro.*

The volatile oil was produced by water vapor distillation approach. The volatile oil of temu kunci rhizoma is formulated by three basic ointment types, namely, water-soluble base, Hydrophilic Base (USP) and *Vanishing cream* base with variations of 3% $^{v}/_{b}$, 6% $^{v}/_{b}$, 9% $^{v}/_{b}$, and 12% $^{v}/_{b}$. Anti-fungus activity was tested by diffusion method, measuring inhibitive area diameter on *Candida albicans* fungus growth. The tests of physical characteristic of volatile oil ointment included tests of viscosity, stickiness, and distribution of ointment. The results of ointment-physical characteristic and anti-fungus activity tests were analyzed by anova and followed up by t-test (*Scheffe* test) with 95% reliance rate.

Obtainable results of anti-fungus activity indicated that the higher concentration of volatile oil of temu kunci rhizoma, the bigger inhibitive diameter is. In the anti-fungus activity test, the best anti-fungus activity was 12% $^{v}/_{b}$ water-soluble base with 2,55 cm inhibitive diameter and the lowest in *Vanishing cream* of 3% $^{v}/_{b}$ with 0,87 cm inhibitive diameter than other ointment base and concentration. In the physical characteristic tests of ointment, the biggest viscosity of 3% $^{v}/_{b}$ water-soluble base was 700 poises and the lowest of *Vanishing cream* base of 12% $^{v}/_{b}$ was 125 poises than other ointment base and concentration. The longest stickiness of 3% $^{v}/_{b}$ water-soluble base was 6 seconds, and the quickest of 12% $^{v}/_{b}$ *Vanishing cream* base was 6,00 seconds than other ointment base and concentration. The widest distribution of 12 $^{v}/_{b}$ *Vanishing cream* base of diameter was 5,70 cm and the narrowest of 3% $^{v}/_{b}$ water-soluble base of diameter was 2,15 cm than other ointment base and concentration.

Key word : Temu kunci rhizoma, ointment

A. INTRODUCTION

Candida albicans is opportunistic pathogenic fungus that is most often met on human being. As normal flora, but in a certain condition, it can grow excessively and invading. *Candida albicans*-caused infection is one of skin infection. Skin infected by this fungus will appear to be red, emits fluids, has smooth scales, itching and painful as well as forms vesicle (Jawetz et.al., 1996).

Ointment is semi-compact form purposed for topical usage on skin or mucous membrane (Anonym, 1995). Ointment may not have rancid smell. Except it is stated another, medicinal content in the ointment that contains narcotic medicine is 10% (Anonym, 1979).

Temu kunci rhizoma contains volatile oil (cineol, camphor, d-borneol, d-pinen sescuiterpen, zingiberen, curcumin, zedoarin (Muhlisah, 1995), saponin and flavanoid (Sugati and Johny, 2001). Volatile oil in the temu kunci rhizoma is useful for anti-fungus (Rediningsih, 2002). Anti-fungus activity of temu kunci rhizoma volatile oil in ointment form is influenced by ointment base type and the volatile oil concentration.

Anti-fungus activity test of temu kunci rhizoma volatile oil was performed in this research with ointment base variation, namely, water-soluble base, Hydrophilic Base and *Vanishing cream* base with volatile oil content variation of 3% $^{v}/_{bs}$, 6% $^{v}/_{bs}$, 9% $^{v}/_{bs}$ and 12% $^{v}/_{bs}$.

B. RESEARCH METHOD

1. Research Material and Tools

a. Materials used in this research are:

Temu kunci rhizoma, PEG 4000, Stearil alcohol, Glycerine, Natrium Laurile Sulphate, Distillated water, Propilenglicol, White Vaseline, Distillated water, Stearic acid, White wax and TEA, *Candida albicans*, 0.9% sterile NaCl solution, SDA (*Sabaroud's Dextrose Agar*) Medium, Sterile aquadest and 2% Miconazole cream.

b. Tools used in this research are:

Stahl distillation, steam destillation and mortar water, stanper, water vaporizer, porcelain dishes, glass tools, analitic scale *AT 2141*, sterile ointment pot, scaled pipette, micro pipette, propinete, spiritus lamp, sterile palm leaf rid cotton, sterile ose, sterile petric dishes, sterile reaction tube and shelf, yellow tip, blue tip, hole maker (diameter of 5 mm), incubator, ABBE refractometre, *Viscotester Rion VT ¾*, extensometer, weight and a set of ointment adhesive test tools.

2. Research Method

a. Volatile oil ointment production

Production of temu kunci volatile oil ointment with oil concentration variation of 3% $^{v}/_{bs}$, 6% $^{v}/_{bs}$, 9% $^{v}/_{bs}$ and 12% $^{v}/_{bs}$ in 10 gram ointment. 2% Miconazole was used as positive control.

Table 1. Formulation of Temu Kunci Volatile Oil Ointment in Water Soluble Base

Concentration ($^{v}/_{b}$)	Volatile oil	Formula 10 g base				
		PEG 4000	Stearile alcohol	Glycerine	Distillated water	Na. Laurile Sulphate
3%	0,3 ml	2,0 g	3,4 g	3,0 g	1,5 g	0,1 g
6%	0,6 ml	2,0 g	3,4 g	3,0 g	1,5 g	0,1 g
9%	0,9 ml	2,0 g	3,4 g	3,0 g	1,5 g	0,1 g
12%	1,2 ml	2,0 g	3,4 g	3,0 g	1,5 g	0,1 g

Way of production according to Martin, 1961:

1) PEG 4000, stearile alcohol and glycerine are boiled in porcelain dishes at 75°C (phase I)
2) Na. Laurile sulphate is dissolved in distillated water and then boiled at 75°C (phase II)
3) Phase I is added to mortar contained phase II, stir it until it is homogenous
4) Volatile oil of temu kunci rhizoma is added to a part of ointment base (c), stir it until it is homogenous, then add the ointment once again until the ointment mass is 10 grams.
5) Ointment is poured into the tube

Table II. Formulation of Temu Kunci Rhizoma Volatile Oil Ointment in Hydrophilic Base

Concentration (v/b)	Volatile oil	Formula 10 g base				
		Na. Laurile Sulphate	PG	Stearile alcohol	White vaseline	Distillated water
3%	0,3 ml	2,0 g	3,4 g	3,0 g	1,5 g	3,7 g
6%	0,6 ml	2,0 g	3,4 g	3,0 g	1,5 g	3,7 g
9%	0,9 ml	2,0 g	3,4 g	3,0 g	1,5 g	3,7 g
12%	1,2 ml	2,0 g	3,4 g	3,0 g	1,5 g	3,7 g

The way of production according to Martin, 1961:

1) Stearile alcohol and white vaseline are melted on the water vaporizer in porcelain dishes at 75°C
2) Natrium laurile sulphate and propilenglicol are solved in the water, then added to mixture (a) and boil it at 75°C
3) Mixture (b) is stirred until it is frozen
4) Temu kunci volatile oil is added to a part of ointment base (c), stir it until it is homogenous, then add the ointment again until the ointment mass is 10 grams.
5) The ointment is poured into the tube

Table III. Formulation of Temu Kunci Volatile Oil Ointment in *Vanishing Cream*

Concentration (v/b)	Volatile oil	Formula 10 g base				
		stearic acid	White wax	white vaseline	TEA	PG
3%	0,3 ml	1,5 g	0,2 g	0,8 g	0,15 g	6,55 g
6%	0,6 ml	1,5 g	0,2 g	0,8 g	0,15 g	6,55 g
9%	0,9 ml	1,5 g	0,2 g	0,8 g	0,15 g	6,55 g
12%	1,2 ml	1,5 g	0,2 g	0,8 g	0,15 g	6,55 g

The way of production according to Martin, 1961:

1) White wax, stearic acid and white vaseline in the porcelain dishes are boiled on the water vaporizer
2) Tri ethanol amine (TEA) is dissolved in the hot water, add propilenglicol and add them into melt (a), stir it until the ointment mass is 10 grams
3) Temu kunci volatile oil is added to a part of ointment base (b), stir it until it is homogenous, then add the ointment again until the ointment mass is 10 grams
4) The ointment is poured into the tube

b. Anti-fungus activity test

Candida albicans suspension that has same concentration with Mc. Farland standard (10^8 CFU/ml) is taken for 10 µl then pour and scatter it by using sterile rid cotton on the SDA medium, so it has same thickness, then the holes are made on the 5 mm diameter medium.

Ointment that contains temu kunci volatile oil and 2% Miconazole cream (positive control) are poured into the holes of SDA medium in the petric dishes in each ointment base concentration, then incubated for 18-24 hours at 37°C. Look at the bacterial growth and measure the restriction diameter.

c. Physical characteristic evaluation of ointment form

1) Ointment viscosity

Viscostester Rion VT ¾ is prepared at horizontal position and rotor is set so that the pointer needle is in proper position. 15 gr ointment is put in cup viscotester. The rotor is dyed into the ointment up the limit labelled in the rotor. The viscotester is switched on and rotor will begin to be rotated, let it for a moment until the pointer needle is stable. The viscosity rate is calculated in poise scale.

2) Ointment adhesive power

A half gram of ointment is put on the glass object with a certain wide, then covered by other glass object, pressed by 1 kg weight for 5 minutes. Glass object is set on test tool, released by 80 grams weight and record the time required to separate the two objects. Replication is conducted for 3 times for all ointment formulation.

3) Ointment scatter ability

A half gram of ointment is put on the middle of circular glass have scale, then other circular glass which have been deliberated to be to be put on the ointment and let during 5 minute, measure and record ointment diameter disseminating then above glass adding burden again as heavy as 50 gram, hushed during 1 minute and record ointment diameter disseminating. Test continued by enhancing burden as heavy as 50 gram until constant obtained diameter. Replication is conducted for 3 times for all ointment formulation

C. RESULT AND DISCUSSION

1. Result of Anti-fungus Activity Test

To see the release of active compound of temu kunci volatile oil from ointment, microbiological test is conducted by agar diffusion method. This method is chosen because it has benefit such as: it is easy to observe and measure restriction zone from volatile oil, the testing material is easier to interact with fungus, it is more sensitive. In anti-fungus activity test, the medium used is SDA medium, 2% Miconazole is used as positive control, and ointment base without volatile oil is used as negatife control.

Table IV. Restriction Zone Diameter of Temu Kunci Volatile Oil in Various Ointment Base and Concentration

Base	Concentration (%)	N	Restriction zone means ± SD (cm)
Water Soluble	Control (+)	3	2,60 ± 0,05
	Control (—)	3	—
	Ointment 3%	3	1,43 ± 0,04
	Ointment 6%	3	1,68 ± 0,03
	Ointment 9%	3	1,86 ± 0,10
	Ointment 12%	3	2,30 ± 0,20
Hydrophilic	Control (+)	3	2,55 ± 0,10
	Control (—)	3	—
	Ointment 3%	3	1,05 ± 0,05
	Ointment 6%	3	1,33 ± 0,10
	Ointment 9%	3	1,50 ± 0,05
	Ointment 12%	3	1,70 ± 0,05
Vanishing cream	Control (+)	3	2,43 ± 0,08
	Control (—)	3	—
	Ointment 3%	3	0,87 ± 0,03
	Ointment 6%	3	1,13 ± 0,08
	Ointment 9%	3	1,23 ± 0,03
	Ointment 12%	3	1,45 ± 0,10

Explanation:

Control (+) : 2% Miconazole Cream

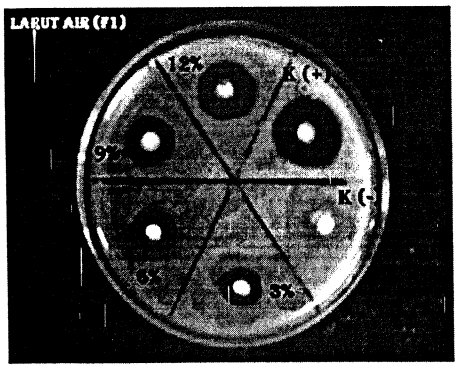
Control (-) : Ointment Base Without Volatile Oil

N : Replication Number

(-) : There is no restriction

From the all three bases used, base that shows restriction to *Candida albicans* from the best rank is water-soluble base, hydrophilic base and *Vanishing cream*. Because in the water-soluble base the comparison between water amount and emulgator (PEG 4000 and Na. laurile acetate) is small, the ability to emulate volatile oil and ointment base is very weak so that the interface stress between volatile oil and ointment base is big, therefore the affinity between volatile oil and ointment base is very small. It causes the volatile oil is easily diffused in the medium and produces largest restriction zone rather than other base.

Result of the research on temu kunci volatile oil activity with water-soluble base is seen in the picture 1.



Explanation:

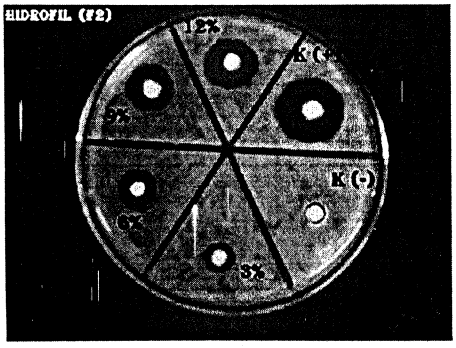
K (+) : 2% Miconazole cream

K(-) : ointment base without volatile oil

Picture 1. Restriction zone of Temu Kunci Volatile Oil in the Water-Soluble Base in Various Concentration.

Hydrophilic base has smaller restriction zone than water-soluble base, but it is larger than *Vanishing cream*. It is because the hydrophilic base contains more component or water phase than its oil phase (vaseline), therefore the interface stress between volatile oil and hydrophilic base is relatively big so that the affinity between oil and hydrophilic base is also big. The volatile oil that is diffused into medium is relatively small rather than water-soluble base and yields wider restriction zone.

Result of the research on temu kunci volatile oil activity is as seen in the picture 2.



Explanation:

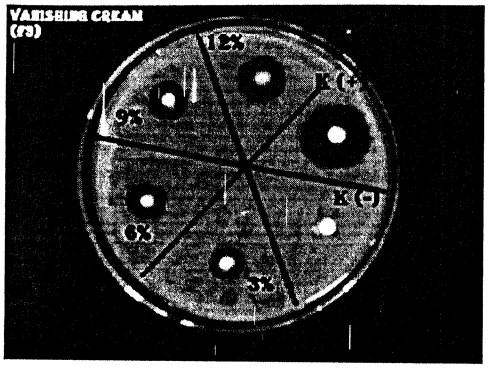
K (+) : 2% Mikonazole cream

K(-) : ointment base without volatile oil

Picture 2. Restriction zone of Temu Kunci Volatile Oil Ointment in Hydrophilic Base in Various Concentration.

Vanishing cream base has narrowest restriction zone toward Candida albicans. Because *Vanishing cream* base is emulsion type with more volatile oil component, namely, stearic acid, white vaseline and cera alba, therefore the interface stress of volatile oil and ointment base is very small and affinity between volatile oil and ointment base becomes big, therefore the volatile oil that can be diffused in SDA medium is very small and yields narrowest restriction zone.

Result of the research on temu kunci volatile oil ointment activity of hydrophilic base is as seen in followed Picture 3:



Explanation:

K (+) : 2% Mikonazole cream

K(-) : ointment base without volatile oil

Picture 3. Restriction Zone of Temu Kunci Volatile Oil Ointment in the Vanishing Cream Base in Various Concentration.

SPSS analysis by two-way ANOVA method and continued with Mann Whitney test were conducted to know the influence of ointment base type and variation of temu kunci volatile oil concentration in each ointment base toward the growth of *Candida albicans* viewed from its α value. If the $\alpha > 0,05$, it means that there is insignificant difference, and if the $\alpha < 0,05$, it means that the difference is significant.

After analyzed by Mann Whitney test, there is significant and insignificant difference between ointment base variation and volatile oil concentration. Mann Whitney test generally shows restriction diameter relation between base type and concentration variation of volatile oil shows significant difference ($\alpha < 0.05$), except relation between positive control of water-soluble base and positive control of hydrophilic base, negative control of water-soluble base with negative control of hydrophilic base and *Vanishing cream* base, water-soluble base of 3%$^{v}/_{b}$ with hydrophilic base of 6%$^{v}/_{b}$, 9%$^{v}/_{b}$ and *vanishing cream* 12%$^{v}/_{b}$, water-soluble base of 6%$^{v}/_{b}$ with hydrophilic base of 12%$^{v}/_{b}$, water-soluble base of 9%$^{v}/_{b}$ with hydrophilic base of 12%$^{v}/_{b}$, water soluble base of 12%$^{v}/_{b}$ with positive control of hydrophilic and positive base of *Vanishing cream*, positive cream of hydrophilic base with positive control of *Vanishing cream*, negative control of hydrophilic base with negative control of Vanishing cream base, hydrophilic base of 3% with *Vanishing cream* base of 12% $^{v}/_{b}$,hydrophilic base of 6% $^{v}/_{b}$ with *Vanishing cream* base of 9% $^{v}/_{b}$ and 12% $^{v}/_{b}$, hydrophilic base of 9% $^{v}/_{b}$ with *Vanishing cream* base of 12% $^{v}/_{b}$, *Vanishing cream* base of 6% $^{v}/_{b}$ with *Vanishing cream* base of 12% $^{v}/_{b}$ ($\alpha > 0.05$). It is because of the ointment ability to restrict the growth *of Candida albicans* on the base and such concentration is almost identical viewed from the restriction standard, therefore there is insignificant difference.

2. Result of Ointment Physical Characteristic Test Evaluation

a. Ointment viscosity

Consistence (viscosity) is not term that is exactly formulated, but only a way to characterize repentance nature. To obtain such characteristic, it needs test by using viscotester on cream form (Voigt, 1995). The smaller the viscosity value of an ointment, the smoother its consistence.

Result of the research shows that Vanishing cream base has lowest viscosity value when compared with hydrophilic base and water-soluble base. Water-soluble base has highest viscosity because there is stearile alcohol in such base that can harden ointment form that contains water and the amount is more than hydrophilic base. The higher the volatile oil concentration in the base, the lower its viscosity, because volatile oil is fluid so it can cause the ointment smoother.

TableV. Viscosity Test of Volatile Oil Ointment in Various Ointment Base and Concentration

Base	Concentration ($^{v}/_{b}$)	N	Viscosity means (poise)
Water Soluble	Ointment 3%	3	700
	Ointment 6%	3	500
	Ointment 9%	3	400
	Ointment 12%	3	300
Hydrophilic	Ointment 3%	3	500
	Ointment 6%	3	400
	Ointment 9%	3	300
	Ointment 12%	3	200
Vanishing cream	Ointment 3%	3	300
	Ointment 6%	3	250
	Ointment 9%	3	150
	Ointment 12%	3	125

Explanation:

N : replication number

SD : Deviation Standard

To know the influence of ointment base and temu kunci volatile oil concentration toward ointment viscosity, SPSS analysis is conducted by two-way ANOVA method and continued with Mann Whitney test. From the Mann Whitney test, it can be concluded that viscosity relation between base types and volatile oil concentration variation shows significant difference ($\alpha < 0.05$), except relation between water-soluble base of 6 % $^{v}/_{b}$ with hydrophilic base of 3% $^{v}/_{b}$, water-soluble base of 9% $^{v}/_{b}$ and hydrophilic base of 6% $^{v}/_{b}$, water-soluble base of 12% $^{v}/_{b}$ and hydrophilic base of 9% $^{v}/_{b}$, water soluble base of 12% $^{v}/_{b}$ and *Vanishing cream* base of 3% $^{v}/_{b}$ and hydrophilic base of 9% $^{v}/_{b}$ and

Vanishing cream base of 3% $^{v}/_{b}$ ($\alpha > 0.05$). It is because of the ointment viscosity on the base and such concentration is equal, therefore the viscosity value is also equal.

b. Ointment Adhesive Power

Adhesive power is ointment ability to be attached to the skin. The better ointment adhesive power, the longer it is attached to the skin, therefore the contact between skin and medicine is also longer and it expected that the volatile oil absorption by the skin is also maximum.

Result of the research shows that water-soluble ointment base has longest adhesive power than hydrophilic and *Vanishing cream* base, because in the water-soluble base the compact component is more than its fluid component, therefore its viscosity is higher. From the four concentrations, the concentration of 12% $^{v}/_{b}$ has smallest adhesive power, because the more volatile oil, the faster the release of the two glass objects. Thus, the bigger the viscosity, the bigger adhesive power.

TableVI. Adhesive Power Test of Volatile Oil Ointment in Various Ointment Base and Concentration

Base	Concentration ($^{v}/_{b}$)	N	Adhesive power means ± SD (second)
Water Soluble	Ointment 3%	3	6,00 ± 0,30
	Ointment 6%	3	4,80 ± 0,17
	Ointment 9%	3	3,90 ± 0,20
	Ointment 12%	3	3,07 ± 0,15
Hydrophilic	Ointment 3%	3	1,67 ± 0,15
	Ointment 6%	3	1,20 ± 0,10
	Ointment 9%	3	1,00 ± 0,10
	Ointment 12%	3	0,83 ± 0,06
Vanishing cream	Ointment 3%	3	1,00 ± 0,10
	Ointment 6%	3	1,00 ± 0,10
	Ointment 9%	3	0,83 ± 0,06
	Ointment 12%	3	0,63 ± 0,06

Explanation:

N : replication number

SD : Deviation Standard

To know the influence of ointment base type and volatile oil concentration toward ointment adhesive power, SPSS analysis is conducted by two-way ANOVA test and then continued with Mann Whitney test. From the Mann Whitney test, it can be concluded that relation of adhesive power between base types and volatile oil concentration shows significant difference ($\alpha < 0.05$), except relation between hydrophilic of 6% $^{v}/_{b}$ and *Vanishing cream* 3% $^{v}/_{b}$. Hydrophil of 9% $^{v}/_{b}$ and hydrophil

12% $^{v}/_{b}$, Vanishing cream 3% $^{v}/_{b}$, 6% $^{v}/_{b}$ and 9% $^{v}/_{b}$, Hydrophil of 12% $^{v}/_{b}$ and *Vanishing cream* 6% $^{v}/_{b}$, 9%, *Vanishing cream* 3% $^{v}/_{b}$ and *Vanishing cream* 6%, and *Vanishing cream* 9% $^{v}/_{b}$ and *Vanishing cream* 6%. It is because ointment in such base and concentration has almost same time or adhesive power, therefore there is very small difference

c. Ointment Scatter Ability

Scatter ability causes ointment base ability to scatter above the skin surface. Ointment is expected to easily scatter without pressure, which means that the wider ointment surface that contacts with the skin, the more medicine contact released. Ability to scatter ointment is influenced by base concentration. Tool used to measure scatter ability of ointment is extensometer, namely, 2 glass in circular form, the one is labelled with scale and the other is closure.

Result of the research shows that *Vanishing cream* base has biggest scatter ability, then hydrophilic base and water-soluble base.*Vanishing cream* base has highest scatter ability because such base contains oil phase (emulsion type), therefore the consistence is smoother and has small viscosity value, therefore it is easier to scatter rather than Hydrophilic and water-soluble base. From various concentration it is seen that the higher concentration of volatile oil in the ointment base, the bigger scatter ability from such ointment base.

TableVII. Scatter Ability Test of Volatile Oil Ointment in Various Ointment Base and Concentration

Base	N	Concentration ($^{v}/_{b}$)	Burden (gr)	Scatter Ability Diameter (cm)
Water Soluble	3	3 %	52,3656	1,73 ± 0,06
			102,3656	2,03 ± 0,03
			152,3656	2,15 ± 0,38
	3	6 %	52,3656	2,00 ± 0,09
			102,3656	2,18 ± 0,08
			152,3656	2,50 ± 0,05
	3	9 %	52,3656	2,13 ± 0,03
			102,3656	2,50 ± 0,09
			152,3656	2,65 ± 0,05
	3	12 %	52,3656	2,48 ± 0,06
			102,3656	2,75 ± 0,09
			152,3656	2,90 ± 0,05
Hydrophilic	3	3 %	52,3656	2,10 ± 0,05
			102,3656	2,60 ± 0,05

			152,3656	2,75 ± 0,17
	3	6 %	52,3656	2,35 ± 0,05
			102,3656	3,07 ± 0,10
			152,3656	3,38 ± 0,06
	3	9 %	52,3656	2,84 ± 0,05
			102,3656	3,40 ± 0,05
			152,3656	3,82 ± 0,03
	3	12 %	52,3656	3,35 ± 0,05
			102,3656	3,90 ± 0,13
			152,3656	4,18 ± 0,03
Vanishing cream	3	3 %	52,3656	3,58 ± 0,08
			102,3656	4,22 ± 0,06
			152,3656	4,73 ± 0,03
	3	6 %	52,3656	4,20 ± 0,05
			102,3656	4,62 ± 0,08
			152,3656	5,17 ± 0,08
	3	9 %	52,3656	4,53 ± 0,10
			102,3656	5,25 ± 0,05
			152,3656	5,48 ± 0,13
	3	12 %	52,3656	5,02 ± 0,15
			102,3656	5,38 ± 0,03
			152,3656	5,70 ± 0,05

Explanation:

N : replication number

(52.3656) : heavy of cover glass

To know the influence of ointment base type and volatile oil concentration of temu kunci rhizome toward ointment scatter ability, SPSS analysis is conducted by two-way ANOVA method and then continued with *Scheffe test*. From the *Scheffe test*, it can be concluded that relation of ointment scatter ability (weight of 152,3656 grams) between base type and volatile oil concentration variation shows significant difference ($\alpha < 0.05$), except relation between water-soluble 3% $^{v}/_{b}$ and water soluble 6% $^{v}/_{b}$, water-soluble 6% $^{v}/_{b}$ and water-soluble 9% $^{v}/_{b}$, water-soluble 9% $^{v}/_{b}$ and water-soluble 12% $^{v}/_{b}$ and hydrophil 3% $^{v}/_{b}$ and water-soluble 12% $^{v}/_{b}$ and hydrophil 3% $^{v}/_{b}$ and Vanishing cream 9% $^{v}/_{b}$ and Vanishing cream 12% $^{v}/_{b}$ ($\alpha < 0.05$). It is because the ointment in such base and concentration has almost equal scatter ability; therefore the diameter of scatter ability is almost equal. Thus, there is insignificant and very small difference.