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Original article

Nanoemulgel Formulation with a Combination of N-Butanol Extract of *Centella asiatica*, N-Butanol Extract of *Sapindus rarak* and Neem Seed Oil

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ABSTRACT:
Spermicide gel containing the active ingredient nonoxynol-9 is one of the non-hormonal contraceptives in the form of a gel that is widely used among the public, however, nonoxynol-9 compounds can cause irritation and itching in the vagina. In this study, alternative spermicidal herbs from *Centella Asiatica*, *Sapindus Rarak* and neem plants were made which were formulated in the nanoemulgel delivery system. In this study, optimization and formulation of spermicide herbal nanoemulgel made from a combination of *Centella asiatica* N-Butanol extract, N-Butanol extract of *Sapindus rarak*, and Neem seed oil were carried out using a full factorial design model (23). In this study, the results were obtained that the particle size of nanoemulgel had a range of 51,9 ± 0,5 to 303,0 ± 3,2 nm. The spreadability of nanoemulgel had a range of 5,8 ± 0,3 to 6,5 ± 0,2 cm. Zeta Potential of nanoemulgel had a range of -9,0 ± 0,0 to -25,0 ± 2,4 mV. The resulting pH range is 5,9 ± 0,2 to 6,2 ± 0,3, the resulting viscosity is 533 ± 0,2 to 681 ± 0,2 cps. The variable concentration of N-Butanol extract of *Sapindus rarak* and the interaction between *Centella asiatica* N-butanol extract and Neem seed oil had a significant effect on the particle size response in terms of the p-value of < 0.05 with p-values of 0.04 and 0.036, respectively. In the zeta potential response and spreadability response, all the variable concentrations of N-Butanol extract of Sapindus rarak, *Cetella asiatica* N-butanol extract, and Neem seed oil; interactions between the n-butanol N-Butanol extract of *Sapindus rarak* and the *Centella asiatica* N-butanol extract; interaction between Centella asiatica N-butanol extract and Neem seed oil; and N-Butanol extract of *Sapindus rarak* and Neem seed oil have a p-value of > 0.05, so it can be concluded that there is no significant effect on zeta potential response and spreadability.
Keywords: *Centella asiatica*, *Sapindus rarak*, Neem, Nanoemulgel, Spermicide.

1. INTRODUCTION

One of the active ingredients of spermicides that are still imported is *Nonoxynol-9*. Spermicide products circulating in the community contain *Nonoxynol-9* (N-9). N-9 negatively affects to epithelial cells, normal vaginal flora, increases vaginal and cervical infections, causes irritation and ulceration, and transmission of HIV/STI (Human Immunodeficience Virus/Sexually Transmitted Infection) [1]. Natural spermicides are a solution because Indonesia is rich in medicinal plants [2]. *Centella asiatica* has anti-spermatogenic, anti-fungal and anti-bacterial properties [3]. The ethanol extract of *Centella asiatica* reduces spermatogenic cells and sperm motility of mice [4]. *Sapindus rarak* contains a lot of saponins. Sapindus rarak can be used to kill all spermatozoa quickly, as well as against Sexually Transmitted Disease caused by Trichomonas vaginalis [5]. *Sapindus rarak* saponin at a dose of 0.5 mg/ml causes sperm inactivation and erosion of the spermatozoa membrane [6]. Neem (Azadirachta indica) has spermicidal activity. Neem leaf extract at a concentration of 200 mg/ml can kill sperm [7].

Nanotechnology-based *nanoemulgels* are a strategy to increase the effectiveness of intravaginal drug delivery related to bio-adhesiveness in vaginal mucus, penetration, better stability and faster release of active ingredients [8]. The intravaginal route was used in the development of spermicides in the form of *nanoemulgels* [7]. *Nanoemulgel* is a preparation that is non-sticky, easy to apply, and comfortable to use [9], with a size of 100-1000 nm [10]. The purpose of this study was to optimize and formulate a spermicidal herbal of *nanoemulgel* made from a combination of n-Butanol extract of *Centella asiatica*, N-Butanol extract of *Sapindus rarak* and Neem seed oil using the response surface full factorial design model , and to perform particle size tests, dispersion tests, pH, viscosity and zeta potential test. Spermicide *nanoemulgel* made from natural ingredients is a new solution and breakthrough in the field of intravaginal spermicide. It is hoped that the spermicidal of

nanoemul gel made from a combination of n-Butanol extract *Centella asiatica*, n-Butanol extract of *Sapindus rarak* and Neem seed oil is proven to have spermicidal activity, is safe, non-toxic, so the formula can replace N-9.

2. METHODS

2.1. Experimental Section

The full factorial design method was used in this study. In this design, three factors are evaluated to obtain the optimal formula. Optimization and formulation in this research design are the concentrations of n-Butanol extract of *Sapindus rarak* (A), n-Butanol extract of *Centella asiatica* (B) and Neem seed oil (C) see table 1. Optimization of this formula aims to obtain optimal results on the independent variables namely particle size (Y1), zeta potential (Y2), spreadability (Y3).

Table 1: Design of Spermicide Nanoemulgel Formula Optimization usingFull Factorial Design Model 2^3

Independent Variable	% (b/v)Concentration		*Coded Values*	
	Low	High	Low	High
A = n-Butanol extractof Sapindus rarak Concentration	1,0	2,5	-1	+1
B = n-Butanol extractof Centella asiatica Concentration	6,5	9,5	-1	+1
C= Neem seed oil Concentration	0,5	2,5	-1	+1

Table 2: Spermicide *Nanoemulgel* Formula with Model Full Factorial Design 2^3

Component	Formula (%)							
	I	II	III	IV	V	VI	VII	VIII
n-Butanol extract of Sapindus rarak	2,5	2,5	1,0	1,0	1,0	1,0	2,5	2,5
n-Butanol extract of Centella asiatica	9,5	9,5	9,5	6,5	6,5	9,5	6,5	6,5
Neem seed Oil	2,5	0,5	0,5	2,5	0,5	2,5	0,5	2,5
Carbopol 940	2	2	2	2	2	2	2	2
Trietanolamin	0,15	0,15	0,15	0,15	0,15	0,15	0,15	0,15
IPM (Isopropyl Myristate)	5	5	5	5	5	5	5	5
Span 80	5,1	5,1	5,1	5,1	5,1	5,1	5,1	5,1
Propilenglycol	10	10	10	10	10	10	10	10
Tween 80	25	25	25	25	25	25	25	25
Metil Paraben	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01
Distilled Water	ad 100	ad 100	ad 100	ad 100	ad 100	ad 100	ad 100	ad 100

2.2. Materials and Instruments

Instruments and tools used in this research are Spectrophotometer UV-Vis Shimadzu, Ultra Turrax Homogenizer, Analitical Balance of Ohaus, Malvern Zetasier, Spreadability Tester, pH Meter L-AQUA, Cone and Plate Viscometer Type CAP 1000 Brookfield.

The materials used in this research were *Centella asiatica*, *Sapindus rarak*, Neem, 96% ethanol, diethyl ether, n-Butanol (Sinopharm Chemical), coconut oil (Sinopharm Chemical), Tween 80 (Solvay Chemicals International), Span 80 (Solvay Chemicals). International) Methyl paraben (Merck), Sodium Metabisulfite (Merck), Triethanolamine (Merck), Carbopol 940 (Merck) with pharmaceutical grade purity.

2.3. Research Procedure

2.3.1. Extraction dan Fractination of N-Butanol of *Centella asiatica, Sapindus rarak* dan Seed Neem Oil

Pericarpium of *Sapindus rarak* and *Centella asiatica* (leaves and petioles), each dried in an oven at 60^0C until a constant weight was obtained and then grinded to obtain a dry powder. Each dry powder was extracted by maceration method using 90% ethanol, shaking every 2 hours for 24 hours. Concentration of the filtrate using a rotary evaporator vacuum. Concentrated extract was made into suspension using distilled water, washed with diethyl ether 1:1, shaken to form 2 phases. The water phase was taken and extracted with n-butanol in a 1:1 ratio. The n-butanol layer was removed by a rotary evaporator. The n-butanol fraction was weighed for each *Sapindus rarak* Extract and *Centella asiatica* Extract. The N-butanol fraction was prepared using 0.9% NaCl at concentrations of 200, 400, and 600 g/mL. Neem seeds are ground and steamed for 30 minutes, pressed until crude oil is produced. Neem seed oil was heated to 70^0C for 15 minutes, added 2% activated charcoal, then stirred and filtered. The oil is heated to 99^0C then 2 drops of phosphoric acid are added. The filtrate was heated for 15 minutes then 3% bleaching earth was added then filtered.

2.3.2. Formulation of *Nanoemulgel* of N-Butanol Extract of *Sapindus rarak*, N-Butanol Extract of *Centella asiatica* and Neem Seed Oil

Methyl paraben and sodium metabisulfite were dissolved in distilled water, and then sprinkled with Carbopol 940 until completely dispersed, then dripped with TEA (Triethanolamine) then stirred and a gel base was formed. In the oil phase, *Centella asiatica* n-butanol extract, n-Butanol extract of *Sapindus rarak*, Neem seed oil are homogenized. In this phase Span 80 is added, VCO (virgin coconut oil) is added. In the water phase, methyl paraben and Na bisulfite were dissolved in distilled water, then heated and tween 80 was added. The water phase was added to the oil phase to form a thick emulsion mass. The formed phase was put into mass 1 (base gel) with a ratio of 45:55, homogenized to form *nanoemulgel* with ultra turax homogenizer at 4000 rpm for 15 minutes.

2.3.3. Physicochemical Characteristics of *Nanoemulgel*

Particle size and zeta potential measurements were carried out using the Particle Size Analyzer (PSA) Zetasizer Malvern series. Samples were weighed weighing 1 gram, added 10 ml of distilled water, homogenized. A sample of 1.5 ml was put into the Particle Size Analyzer cuvette .

2.3.4. pH measurement using a standardized pH meter using phosphate buffer with a pH of 7.4 using the L-AQUA pH meter instrument

2.3.5. Measurement of the viscosity of the *nanoemulgel* samples, using a Brookfield cone and plate viscometer type CAP 1000 with spindle number No. 40, the sample cup is

Tabel 3: Results of Physicochemical Characteristics of Herbal Spermicidal *Nanoemulgel*

Formula	Particle Size± SD (nm)	Polydispersity Index ± SD (PDI)	Zeta Potential ± SD (mV)	Viscosity± SD (cps)	Spread-ability± SD (cm)	pH ± SD
F I	156,4± 0,1	0,563± 0,001	-16,8± 1,3	615± 0,2	6,7± 0,1	6,2 ± 0,3
F II	148,5± 1,4	0,533± 0,007	-14,6± 0,1	673± 0,3	6,1 ± 0,2	6,2 ± 0,2
F III	94,4± 0,6	0,370± 0,002	-16,5± 1,3	533 ± 0,2	5,9 ± 0,2	6,1 ± 0,0
F IV	71,1 ± 0,5	0,375± 0,003	-16,2± 2,7	590± 0,9	5,8± 0,3	6,2 ± 0,2
F V	59,6± 0,2	0,432 ± 0,000	-17,0± 0,6	681± 0,2	6,3± 0,1	6,1 ± 0,1
F VI	303,0± 3,2	0,413± 0,004	-10,3± 0,1	587± 0,2	6,5± 0,2	5,9 ± 0,2
F VII	51,9± 0,5	0,234 ± 0,029	-25,0± 2,4	550± 0,9	6,1± 0,2	6,2 ± 0,0
F VIII	106,5± 0,1	0,376± 0,015	-9,0± 0,0	671± 0,2	6,2± 0,3	6,2 ± 0,2

mounted on the cone viscometer at a speed of 10 rpm.

2.3.6. Spreadability test

The preparation is weighed at 50 mg and then placed on a glass plate for dispersion testing, with duration of 1-2 minutes. Measure the diameter of the distribution using a caliper, with 3x replication [11].

Data analysis

Statistical analysis was performed DOE (Design of Experiment) Full Factorial Design with Minitab software version 16. The independent variables in this research design were n-Butanol extract of *Sapindus rarak* (XA), n-Butanol Extract *Centella asiatica* (XB) and Neem seed oil (XC). The dependent variables include particle size (Y1), zeta potential (Y2), scattering power (Y3). The multiple linear regression method was used to analyze the effect and to predict the independent variables, namely A and B on the dependent variable, namely Y. To calculate b0, b1, and b2, it can be used the Least Square Method which produces the following equation [11].

$b0 + b1\sum X_A + b2\sum X_B + + b3\sum X_C = \sum Y$

Description:

b0 = independent variable coefficient value

b1 = coefficient value ofXA

b2= coefficient value of XB

b3= coefficient value ofXC

3. RESULTS AND DISCUSSION

In the preparation of *nanoemulgel* with a combination of N-Butanol extract of *Sapindus rarak* (A), n-Butanol extract of *Centella asiatica* (B) and Neem seed oil (C), it was found that the particle size of spermicidal herbal *nanoemulgel* has a range of 477.5 ± 0.2 to 781 .2 ± 0.6nm. The spreading power of the Spermicide Herbal *Nanoemulgel* ranges from 5.8 ± 0.2 to 6.6 ± 0.1 cm. The Zeta Potential of Herbal Spermicide *nanoemulgel* has a range of -37.1 ± 0.7 to -45.9 ± 0.8 mV. The resulting pH range is 5.9 ± 0.1 to 6.6 ± 0.1, the resulting viscosity is 768 ± 0.8 to 833 ± 0.8 cps, as shown in Table 3 below.

The particle size of the spermicidal herbal *nanoemulgel* covers the range of less than 1000 nm. The magnitude of the effect of each factor individually or in interaction can be seen in Table 4. A positive (+) or negative (-) sign indicates whether the effect of the factor increases (+) or decreases (-) the response. From Table 4 it can be seen that the factor of n-Butanol extract of *Sapindus rarak* (A) and the n-Butanol extract of *Centella asiatica* and Neem seed oil (BC) had an effect on increasing the particle size response.

Table 4: Effect Value on Particle Size Parameter Response

Formula	Factor	Effect
A	n-Butanol extract of Sapindus rarak	0,26
B	n-Butanol extract of Centella asiatica	-1,68
C	Neem seed oil	-1,16
AB	n-Butanol extract of Sapindus rarak* n-Butanol extract of Centella asiatica	-0,49
AC	n-Butanol extract of Sapindus rarak* Neem seed oil	-0,65
BC	n-Butanol extract of Centella asiatica* Neem seed oil	0,62

The influence of the factors of the n-Butanol extract of *Sapindus rarak*, n-Butanol extract of *Centella asiatica* and Neem seed oil, as well as the interaction between each other, can be seen in the following graph.

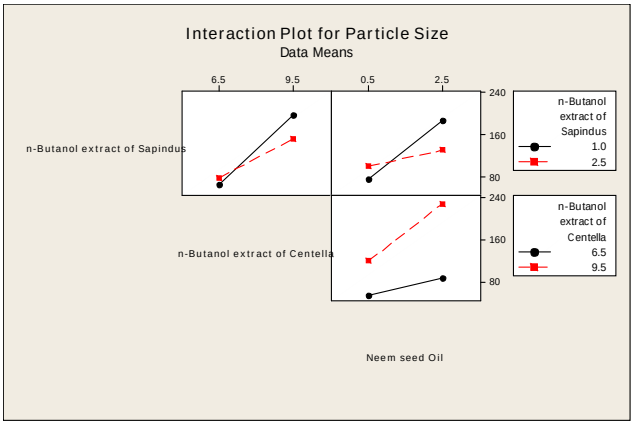

Fig 1: The Effect of the concentration of N-Butanol extract of Sapindus rarak (A), N-Butanol Extract of Centella asiatica (B), Neem seed oil (C) on Particle Size Response

Based on Figure 1, it can be seen that the effect of increasing the n-Butanol extract of Sapindus rarak concentrations of 1.0% and 2.5% with n-Butanol extract of Centella asiatica would decrease the particle size response. The effect of the n-Butanol *Sapindus rarak* extract at a concentration of 1.0% with neem seed oil increased the particle size response while an increase in the concentration of 2.5% n-Butanol *Sapindus*

rarak extract with neem seed oil tends to increase the particle size. The effect of increasing the n-Butanol extract of Centella asiatica at a concentration of 6.5% and 9.5% with neem seed oil will increase the particle size response. Increasing the concentration of the liquid phase tends to reduce particle size due to increased mobility of the lipid phase [12][13].

Table 5: Coefficient and *P-Value*Particle Size Response

	Coefficient	*P-value*
Constant	123,80	0,154
A	7,97	0,838
B	-51,52	0,341
C	-35,45	0,453
AB	-14,90	0,712
AC	-19,83	0,634
BC	18,93	0,647

It can be seen from Table 5 that the equation for the particle size response can be obtained as follows:

$$Y1 = 123{,}80 + 7{,}97X_A - 51{,}52X_B - 35{,}45X_C - 14{,}90X_{AB} - 19{,}83X_{AC} + 18{,}93X_{BC}$$

As it can be seen in Table 5 that the significance of the equation model for the particle size response as the single significance or the interaction between factors in influencing the particle size response. If the p-value is less than 0.05 this has a significant meaning, whereas if the p-value is more than 0.05 it means it is not significant. The equation model for the particle size response has a p-value greater than 0.05, which is 0.154, which means it is not significant but has a R square value (model goodness) of 84.08% so that the equation model for the particle size response can be used to predict the particle size response.

All factors, the n-Butanol extract of *Sapindus rarak* (A), n-Butanol extract of *Centella asiatica* (B), Neem seed oil (C), interactions of n-Butanol extract of *Sapindus rarak* and n-Butanol extract of *Centella asiatica* (AB), the interaction between n-Butanol extract of *Sapindus rarak* and Neem seed oil (AC) and interactions between extract of n-Butanol Centella asiatica and Neem Seed Oil (BC) has a p-value greater than 0.05. So that individually did not significantly affect the particle size response.

Based on data analysis using a factorial design, factors of n-butanol extract of *Sapindus rarak*, n-Butanol extract of *Centella asiatica* and Neem seed oil, as well as interactions between each other have an effect on the resulting zeta potential response. From Table 6, it can be seen that the concentration factors of n-Butanol extract of *Sapindus rarak* (A) and the interaction between extract of n-Butanol *Sapindus rarak* and Neem seed oil (AC) have the effect of increasing the zeta potential response.

Tabel 6: Effect Value on Zeta Potential Response

Formula	Factor	Effect
A	n-Butanol extract of Sapindus rarak	0,24
B	n-Butanol extract of Centella asiatica	-0,39
C	Neem seed oil	-0,88
AB	n-Butanol extract of Sapindus rarak* n-Butanol extract of Centella asiatica	-0,15
AC	n-Butanol extract of Sapindus rarak* Neem seed oil	0,29
BC	n-Butanol extract of Centella asiatica* Neem seed oil	-0,54

The influence of the factors of N-Butanol extract of *Sapindus rarak*, n-Butanol extract of *Centella asiatica* and Neem seed oil, as well as the interaction between each other can be seen in the following graph.

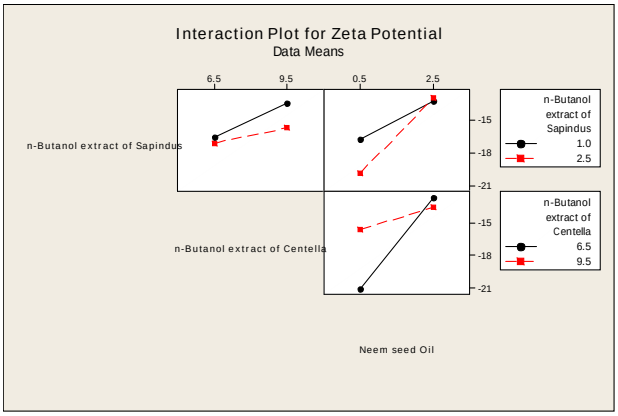

Fig 2: Effect of N-Butanol extract of Sapindus rarak Concentration (A), N-Butanol Extract of Centella asiatica (B), Neem Seed Oil (C) on Zeta Potential Response

Based on Figure 2, it can be seen that the effect of increasing the n-butanol extract of *Sapindus rarak* with the n-butanol extract *Centella asiatica* will decrease the zeta potential response. The effect of increasing the n-butanol extract of *Sapindus rarak* with Neem seed oil will increase the zeta potential while the effect of increasing the n-butanol extract of *Centella asiatica* at concentrations of 6.5% and 9.5% with Neem seed oil concentrations of 0.5% and 2.5% will reduce the zeta potential response. Table 3 shows that zeta potential values greater than -30 mV, it indicates good stability for each of these spermicidal herbal *nanoemulgel* formulations. A zeta potential value below ±10 mV indicates low stability of the preparation, resulting in attraction between the particles which causes the particles to melt together [14].

Table 7: Coefficient and P-Value Respon Zeta Potential

	Coefficient	P-value
Constant	-15,70	0,118
A	0,70	0,852
B	-1,15	0,763
C	-2,60	0,540
AB	-0,45	0,904
AC	0,85	0,821
BC	-1,60	0,684

It can be seen from Table 7 that the equation for the zeta potential response as follows:

$$Y2 = -15{,}70 + 0{,}70X_A - 1{,}15X_B - 2{,}60X_C - 0{,}45X_{AB} + 0{,}85X_{AC} - 1{,}60X_{BC}$$

In Table 7 shows that the significance of the equation model for the zeta potential response as well as the single significance or the interaction between factors in influencing the zeta potential response. If the p-value is less than 0.05 this has a significant meaning, whereas if the p-value is more than 0.05 it means it is not significant. The equation model for the zeta potential response has a p-value greater than

0.05, which is 0.118, which means it is not significant but has a R square value (model goodness) of 58.08% so that the equation model for the zeta potential response can be used to predict the zeta potential response.

All factors, i.e. extract of n-Butanol *Sapindus rarak* (A), extract of n-Butanol *Centella asiatica* (B), Neem seed oil (C), interactions between extract of n-Butanol *Sapindus rarak* and extract of n-Butanol *Centella asiatica* (AB), interactions between extract of n-Butanol *Centella asiatica* and Neem Seed Oil (BC) and the interaction between extract of n-Butanol *Sapindus rarak* and Neem seed oil (AC) has a p-value greater than 0.05, so that individually does not have a significant effect on the zeta potential response.

Based on data analysis using a factorial design, the factors of n-Butanol extract of Sapindus rarak, extract of n-Butanol *Centella asiatica* and Neem seed oil, as well as interactions between each other have an effect on the resulting spreadability response. From Table 8 it can be seen that the interactions of n-Butanol extract of *Sapindus rarak* and n-Butanol extract of *Centella asiatica* (AB), the interaction between n-Butanol extract of *Sapindus rarak* and Neem seed oil (AC) and interactions between extract of n-Butanol Centella asiatica and Neem seed oil (BC)has the effect of increasing the spreadability response.

Table 8: Effect Value on Spread Response

Formula	Factor	Effect
A	n-Butanol extract of Sapindus rarak	-1,00
B	n-Butanol extract of Centella asiatica	-1,33
C	Neem Seed Oil Seed	-1,33
AB	n-Butanol extract of Sapindus rarak* n-Butanol extract of Centella asiatica	0,33
AC	n-Butanol extract of Sapindus rarak* Neem seed oil	1.00
BC	n-Butanol extract Centella asiatica* neem seed oil	2,67

The influence of the factors of n-Butanol extract of *Sapindus rarak*, n-Butanol extract of *Centella asiatica* and Neem seed oil, as well as the interaction between one another can be seen in the following graph.

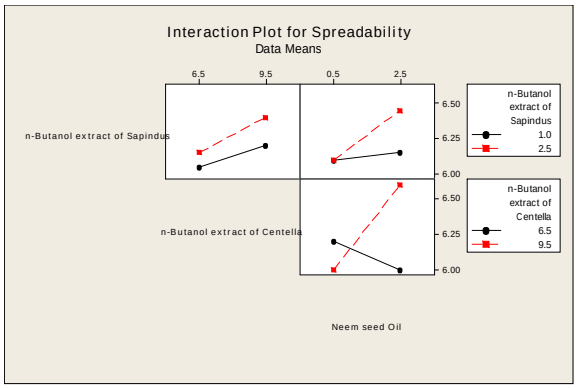

Fig 3: Effect of Interaction on Power Response Spread

Based on Figure 3, it can be seen that the effect of the n-Butanol extract of *Sapindus rarak* with the n-Butanol extract of *Centella asiatica* will increase the spreadability response. In the effect of increasing n-Butanol *Sapindus rarak* extract at a concentration of 1.0% with neem seed oil had almost no effect the spreading power response while n-Butanol extract of *Sapindus rarak* concentration of 2.5% with Neem seed oil will increase the spreading power response. The effect of increasing the n-Butanol extract of *Centella asiatica* at a concentration of 6.5% with Neem seed oil will decrease the spreadability response while the n-butanol extract of *Centella asiatica* at a concentration of 9.5% with Neem seed oil will increase the spreadability response.

Table 9: Coefficientand P-Value of Spread Response

	Coefficient	P-value
Constant	6,200	0,008
A	-0,075	0,500
B	-0,100	0,410
C	-0,100	0,410
AB	0,025	0,795
AC	0,075	0,500
BC	0,200	0,228

From Table 9 it can be obtained the equation for the spreading power response is as follows:

$$Y3 = 6{,}200 - 0{,}075X_A - 0{,}100X_B - 0100X_C + 0{,}025X_{AB} + 0{,}075X_{AC} + 0{,}200X_{BC}$$

In Table 9 it can be seen the significance of the equation model for the scattering response as well as the singular significance as well as the interaction between factors in influencing the scattering response. If the p-value is less than 0.05 this has a significant meaning, whereas if the p-value is more than 0.05 it means it is not significant. The equation model for the scatter response has a p-value smaller than 0.05, which is 0.008, which is significant and has a R square value (model goodness) of 92.74% so that the equation model for the scatter response can be used to predict the scatter response of a process condition at the concentration limit studied.

All factors, i.e. n-Butanol extract of *Sapindus rarak* (A), n-Butanol extract of *Centella asiatica* (B), Neem seed oil (C), interactions between n-Butanol extract of *Sapindus rarak* and n-Butanol extract of *Centella asiatica* (AB), the interaction between n-Butanol extract of *Sapindus rarak* and Neem seed oil (AC) and the interactions between n-Butanol extract of *Centella asiatica* and Neem seed oil (BC) has a p-value greater than 0.05. So that it does not have a significant effect on the spreading power response. The spreadability test aims to measure the spread of the preparation on the skin. A good spread of *nanoemulgel* is 5-7 cm. If the diffusion is too low, it will be relatively difficult for the formulation to spread when applied to the skin [15].

4. CONCLUSIONS

The combination of *nanoemulgel* formula of n-Butanol extract of *Centella asiatica*, n-Butanol extract of *Sapindus rarak* and Neem seed oil has been successfully prepared using a factorial design. The equation model for particle size response, scattering power, and zeta potential has a p-value smaller than 0.05 which means it is significant so that the

equation model can be used to predict particle size response. The concentration variable of the n-Butanol extract of *Sapindus rarak* and the interaction between the n-Butanol extract of *Centella asiatica* and Neem seed oil had a significant effect on the particle size response and spreadability as seen from the p-value <0.05. In the zeta potential response and spreadability response, all concentration variables of n-Butanol extract of *Sapindus rarak*, n-Butanol extract of *Centella asiatica*, Neem seed oil, the interactions of n-Butanol extract of *Sapindus rarak* and n-Butanol extract of *Centella asiatica*, the interactions between n-Butanol extract of *Centella asiatica* and Neem seed oil and the interaction of n-Butanol extract of *Sapindus rarak* and Neem seed oil had a p-value > 0.05, so it can be concluded that there was no significant effect on the zeta potential response.

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