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Evaluation and Antimicrobial Activity of Herbal Nanoemulgel Combination of N-Butanol Extracts of *Centella asiatica* and *Sapindus rarak* and Seed Oil of *Azadirachta indica*

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ABSTRACT

Gel spermicide containing the active ingredient nonoxynol-9 is one of the non-hormonal contraceptive products that is widely used, but the compound can cause vaginal infections, irritation, and ulceration. In this study, spermicidal herbal alternatives were made from the plants *Centella asiatica*, *Sapindus rarak*, and *Azadirachta indica*, formulated in a nanoemulgel delivery system. Nanoemulgel formulation was made from a combination of the n-butanol extracts of *Centella asiatica* and *Sapindus rarak* and *Azadirachta indica* seed oil using the Model Response Surface (MRS) method. Physicochemical characteristics showed that the particle size of the nanoemulgel ranges from 52.53 ± 1.17 nm to 234.57 ± 21.32 nm. The Zeta potential of nanoemulgel ranges from -9.36 ± 0.55 mV to 10.14 ± 0.20 mV. The dispersion of nanoemulgel has a range of 5.9–7.20 cm. The viscosity of the gel ranged from 19.377–19.444 mPas. The pH ranged from 4.89 ± 0.04 to 5.19 ± 0.01. Independent variable concentrations of *Centella asiatica* n-butanol extract, *Sapindus rarak* n-butanol extract, and *Azadirachta indica* seed oil has a p-value > 0.05. The study concluded that there were no significant effects of the extracts combination on particle size response, zeta potential, dispersion, viscosity, and pH of the nanoemulgels.

Keywords: *Azadirachta indica, Centella asiatica, Nanoemulgel, Sapindus rarak, Characterization*

Introduction

Spermicide is a material that can paralyze and kill spermatozoa. Spermicide is said to be ideal if it can inhibit the rapid growth of spermatozoa, is free from the effects of long-term use, does not irritate the vaginal mucosa or penis, and is not toxic. Spermicides are widely used in the form of liquid sprays, creams, and foaming tablets and can be given or added to condoms.[1] One of the spermicidal products in the market still contains nonoxynol-9 (N-9). However, nonoxynol-9 (N-9) can negatively affect epithelial cells, normal vaginal flora, increase vaginal infections, cervix, cause irritation and ulceration, and transmit Human Immunodeficiency Virus and sexually transmitted infections.[2]

Natural spermicides are good alternatives to synthetic agents, and Indonesia is rich in medicinal plants that are readily available.[3] Herbal plants useful as spermicides are gotu kola (*Centella asiatica*)[4], *Sapindus rarak*, and neem seed oil (*Azadirachta indica*). Gotu kola (*Centella asiatica*) contains alkaloids, phytosterones, sesquiterpenes, sterols, and tannins. Gotu kola can be efficacious as a spermatogenic, anti-bacterial, and antifungal.[5]

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According to previous research, giving gotu kola extract as much as 20 mg/kg body weight can act as an antifertility that affects the morphology and motility of spermatozoa.[6]

Sapindus rarak plant (*Sapindus rarak* DC) contains saponins. N-butanol fraction of *Sapindus rarak* fruit at a dose of 200 µg/mL and 400 µg/mL for 1 minute produced spermatozoa with average percentage motility of 17.23% and 4.5%, while at a dose of 600 µg/mL for 1 minute, can cause all spermatozoa to become immotile or die with zero percentage motility.[7]

Neem seeds (*Azadirachta indica*) oil contain active pesticide substances, namely azadirakhtin, which reaches 0.1–0.5% (average 0.25%) of the dry weight of neem seeds. Administration of neem seed ethanol extract with concentrations of 2%, 4%, 8%, and 16% showed decreased spermatozoa activity. Administration of neem seed ethanol extract with a concentration of 16% showed maximum antifertility effect.[8,9] Spermicides containing the extract's active ingredient Nanotechnology-based nanoemulgels are a strategy to improve the effectiveness of intravaginal drug delivery related to biodegradability in the vaginal mucus, penetration, better stability, and faster release of active ingredients. The intravaginal route is used to develop nanoemulgel spermicides with a size of 10–600 nm.[4] In this study, physicochemical characteristics and activity tests of herbal nanoemulgel preparations will be carried out in combination with N-butanol *Centella asiatica* extract, N-butanol *Sapindus rarak* extract, and *Azadirachta indica* seed oil to obtain optimal results with the Model Response Surface (MRS) method.

Materials and Methods

Materials

The instruments and tools used in this research are a rotary evaporator (DLAB RE 100-PRO), a waterbath (DLAB DWB20-S), an Ultra Turrax IKA T25, a particle size analyzer (PSA), a zetasizer (Malvern), a pH

meter (LAQUA), an NDJ 8S viscometer, digital scales, and glassware. Others, materials include Gotu kola powder (Materia Medica), *Sapindus rarak* powder (Materia Medica), Neem Seed Oil (Materia Medica), Tween 80 p.a. (Fisher Chemical), Methyl Paraben p.a. (Fisher Chemical), Isopropyl Myristate p.a. (Fisher Chemical), Propylene Glycol p.a. (Fisher Chemical), Span 80 p.a. (Fisher Chemical), Carbopol 940 p.a. (Fisher Chemical), Triethanolamine p.a. (Fisher Chemical), n-Butanol (Fisher Chemical), Diethyl ether p.a. (Merck), Ethanol p.a. (Fisher Chemical), Distilled water, and Dapar Phosphate pH 7.4.

Plant Collection and Identification

The plants were collected from Materia Medica, Dinas Kesehatan East Java, Batu Indonesia, in October 2022 and authenticated by the Determination Unit of Materia Medica, Dinas Kesehatan East Java, Batu Indonesia. Voucher specimens were deposited at the Department of Pharmacy, Universitas PGRI Adi Buana Surabaya (No. 074/687/102.29-A/2022)

Plant extraction

Extraction Centella asiatica *and* Sapindus rarak

Dry powder of gotu kola and *Sapindus rarak* was extracted using 90% ethanol at a ratio of 1:3 (1 kg of the powder sample in 3 litres of ethanol) for gotu kola and 1:4 (1 kg of the powder sample in 4 litres of ethanol) for *Sapindus rarak*. The mixture was agitated every 2 hours for 24 hours (Gotu Kola) and 48 hours (*Sapindus rarak*) and then filtered separately. The filtrate was concentrated using a rotary evaporator, and a suspension was made using Aquadest. The suspension was extracted with diethyl ether at a ratio of 1:1. The aqueous phase was extracted with n-butanol at a ratio of 1:1. Then, the n-butanol layer was concentrated to dryness at 40°C using a rotary evaporator.[10]

This study used the Response Surface (MRS) Model using 2 levels and 3 variables. This design evaluated three factors to obtain the optimal formula for preparing the nanoemulgel. Optimization and formulation in this research design are the concentrations of n-butanol extract of *Sapindus rarak* (A), n-butanol pegagan (*Centella asiatica*) extract (B), and neem (*Azadirachta indica*) seed oil (C) (Table 1). Optimization of this formula aims to obtain optimal results on independent variables (X), namely particle size (X1) and entrapment efficiency (X2).

Preparation of spermicidal herbal nanoemulgel formula

The first step in formulating the nanoemulgel is preparing gels and emulsions (Table 2). The gel was prepared by dispersing carbopol with aquadest and adding methylparaben and 20 drops of TEA. The mixture was stirred until a gel base was formed. The pH of the base was checked and recorded. In the HDI oil phase, Tween 80, *Sapindus rarak* n-butanol extract, gotu kola (*Centella asiatica*) n-butanol extract, neem (*Azadirachta indica*) seed oil, propylene glycol, and span 80 were stirred with a magnetic stirrer at a speed of 200 rpm. The above mixture was added to the gel base previously formed and stirred until an emulsion mass was formed. Both phases were mixed using an Ultra Turrax homogenizer at a speed of 6000 rpm for 15 minutes until homogeneous.

Physicochemical Characteristics of Nanoemulgel

pH Measurements

pH measurement was done using the L-AQUA pH meter and standardized using phosphate Buffer at a pH of 7.4. The electrode was dipped into the nanoemulgel sample and stirred gently until the pH was stable. The pH meter reading was noted.[11]

Particle Size and Zeta Potential Measurements

Particle size, particle size distribution, and zeta potential were tested with the Malvern Zetasizer Series Particle Size Analyzer (PSA). The sample (1 g) to be analyzed was mixed with 10 mL of aquadest and homogenized. After homogenization, the sample was transferred into the Analyzer cuvette. The required parameters were selected from the instrument menu, and the measurement was run for 10 minutes. The resulting data was the particle size calculated from average fluctuations and light scattering.[12]

Viscosity Measurements

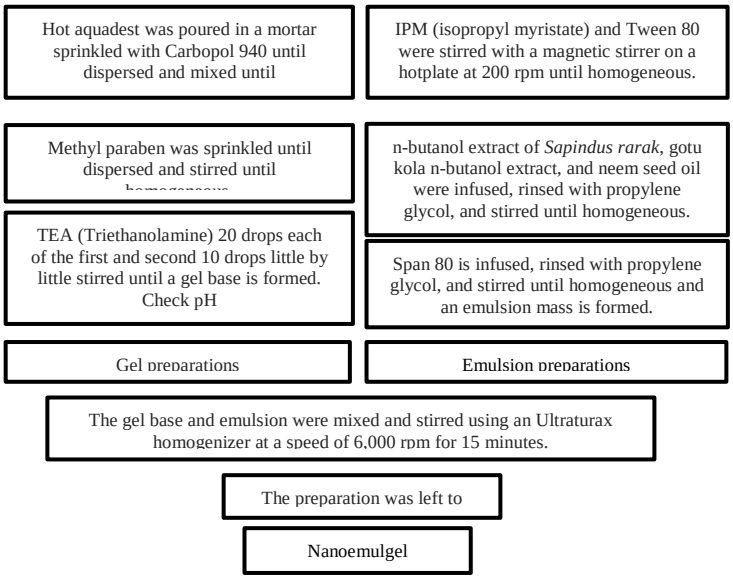
Viscosity testing of nanoemulgel samples was carried out using an NDJ-8S viscometer. The sample was transferred into a glass beaker. The instrument was set to spindle 3 at the speed of 6 rpm. It was allowed to run for some time until the readings on the screen were completed.[14] The viscosity value for nanoemulgel preparations ranges from 4,000–40,000 mPas.[12]

Entrapment Efficiency Measurements

The entrapment efficiency test was carried out by weighing 1000 mg of samples diluted with 10 mL of ethanol p.a. (Asiatic acid), Aquades (azadirachtin), and diethyl ether (diosgenin), then centrifuged for 45 minutes at a speed of 2500 rpm. The resulting sample supernatant levels were obtained. The sample was screened using Millipore 0.45 m, and absorbance was measured using a UV-Vis spectrophotometer. The free content of the active ingredient in the aqueous phase was obtained from the standard absorbance curve regression equation.[13] Entrapment efficiency (EE) was calculated from the equation below:

$$EE = \left(\frac{Wa-Ws}{Wa}\right) \text{x } 100\ \%$$

Wa: Levels of active ingredients added to nanoemulgel
Ws: Levels of untrapped active ingredients (supernatants)



Scheme 1: Spermicidal Herbal Nanoemulgel Manufacturing Scheme.

Table 1: Optimization Design of Nanoemulgel Formula of *Centella asiatica* n-Butanol Extract, *Sapindus rarak* n-Butanol Extract, and Neem Seed Oil Using Surface Response Model 2^3

Independent Variables	% (b/v) Concentration		*Coded Values*	
	Low	High	Low	High
A = n-butanol extract of *Sapindus rarak*	1.0	2.5	-1	+1
B = n-Butanol extract of *Centella asiatica*	5.5	7.0	-1	+1
C = Neem seed oil	0.5	2.5	-1	+1

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Table 2. Formulation of n-Butanol Extracts of *Centella asiatica*, *Sapindus rarak* and Neem Seed Oil Nanoemulgel using a Surface Response Model 2^3

Formula	Formula (%)							
	I	II	III	IV	V	VI	VII	VIII
n-Butanol Extract of *Sapindus rarak*	1	1	2.5	2.5	2.5	1	2.5	1
n-Butanol Extract of Gotu kola	5.5	7	7	5.5	5.5	5.5	7	7
Neem Seed Oil	2.5	0.5	0.5	2.5	0.5	0.5	2.5	2.5
Tween 80	25	25	25	25	25	25	25	25
Methyl Paraben	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
IPM (Isopropyl myristate)	5	5	5	5	5	5	5	5
Propylene Glycol	10	10	10	10	10	10	10	10
Span 80	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.1
Carbopol 940	2	2	2	2	2	2	2	2
Triethanolamine	20 drip	20 drip	20 drip	20 drip	20 drip	20 drip	20 drip	20 drip
Distilled Water	ad 100	ad 100	ad 100	ad 100	ad 100	ad 100	ad 100	ad 100

Determination of Antimicrobial Activity Using Agar Diffusion Method
The determination of the antifungal activity of a spermicidal herbal nanoemulgel combination of Gotu kola (*Centella asiatica*), *Sapindus rarak* n-butanol extract and neem seed oil (*Azadirachta indica*) against *Candida albicans* was carried out by the agar diffusion method using disc paper. The medium was poured into sterile petri dishes and allowed to solidify. After solidifying, as much as 0.1 mL of the fungal suspension was evenly spread over the surface of the medium. Sterile disc papers dripped with test solutions (1000 µg/mL concentration) were placed on the agar medium. The media were incubated at room temperature for three days. Antifungal activity was observed based on the diameter of the inhibitory zone indicated by the clear area formed around the paper disc and measured using a calliper.[14]

Positive Control and Negative Control
The positive control used in the antimicrobial activity test was Relactagel preparation. The negative control was Saburaud Dextrose Agar (SDA) media for *Candida albicans* testing.[15]

Determination of Antimicrobial Activity Against Staphylococcus aureus
The determination of the antibacterial activity of the spermicidal herbal nanoemulgel combination of Gotu kola (*Centella asiatica*), *Sapindus rarak* n-butanol extract, and neem seed oil (*Azadirachta indica*) against *Staphylococcus aureus* was carried out by the agar diffusion method. A 0.1 mL of the bacteria suspension was transferred into a petri dish containing a sterile NA medium. Sterile disc papers dripped with test solutions (1000 µg/mL concentration) were placed on the surface of the agar planted with bacteria and incubated at 37°C for 24 hours. Relactagel was used as a positive control, and Nutrient Agar (NA) media was used as the negative control. Antifungal activity was observed based on the diameter of the inhibitory zone indicated by the clear area formed around the paper disc and measured using a calliper.[16]

Data Analysis
Statistical analysis was performed with the Design of Experiment Response Surface (MRS) with Minitab software version 16.0. The independent variables in this study design were the concentration ratios of *Sapindus rarak* n-butanol extract (A), gotu kola n-butanol extract (B), and neem seed oil (C).

Result and Discussion

An evaluation of the physicochemical characteristics of spermicidal herbal nanoemulgel, including organoleptic tests, particle size, zeta potential, viscosity, and pH, was carried out to compare the characteristics of all nanoemulgel preparations and to determine the effect of differences in the concentration ratio of each extract.

Measurements of physicochemical characteristics of herbal nanoemulgel
The nanoemulgel pH test ranges from 4.89 ± 0.04 to 5.19 ± 0.01. These values meet the pH requirements for topical preparations, which are between 4 and 6 for the skin. In the pH test, there was a decrease in several formulations caused by gotu kola extract, which contains acidic compounds such as Asiatic acid, Brahmic acid, modecasic acid, and various other acids.[17]
The particle size of nanoemulgel ranges from 52.53 ± 1.17 nm to 234.57 ± 21.32 nm. The particle size meets the desired range of 10–600 nm. The smaller the particle size, the easier it is to penetrate the skin membrane and the better the effect. It illustrates that the size of the resulting particles decreases as the amount of extract increases. The particle size of contraceptive vaginal nanoemulgel as an antifungal with the oil phase content of IPM (isopropyl myristate) is 26 nm. In research conducted on topical gel preparations containing *Centella asiatica* and rosemary oil, a particle size of 43.97 ± 5.6 nm was obtained. This particle size is smaller than that in the current study. Therefore, it is necessary to optimize the formula for smaller particle size of the nanoemulgel preparations.[10]
The interaction of gotu kola extract and neem seed oil (BC) decreases particle size response. The factors of *Sapindus rarak* extract (A), gotu kola extract (B), and neem seed oil (C), the interaction of *Sapindus rarak* extract and gotu kola extract (AB), and the interaction of *Sapindus rarak* extract and neem seed oil (AC) have the effect of increasing particle size response. The influence of the factors of *Sapindus rarak* extract, gotu kola extract, and neem oil, as well as the interaction between them, can be seen in Figure 1. From the Figure, it can be seen that increasing gotu kola extract with a *Sapindus rarak* extract by a concentration of 1.0% will decrease particle size response while increasing gotu kola extract with a *Sapindus rarak* extract concentration of 2.5% tends to increase particle size response. The effect of increasing neem seed oil extract with *Sapindus rarak* extract will decrease particle size response. Also, increasing neem seed oil extract at concentrations of 0.5% and 2.5% with gotu kola extract will decrease particle size response.

$$Y = 117{,}37 + 44{,}73X_A + 13{,}35X_B + 20{,}79X_C + 19{,}50X_{AB} + 11{,}07X_{AC} - 4{,}40X_{BC} \ldots. \quad (1)$$

Equation (1) above shows that the significance of the equation model for the particle size response has a p-value > 0.05 (0.066), which means it is insignificant and has an R square value (model goodness) of 95.50% so that the equation model for particle size response can be used to predict the particle size response of a process condition at the concentration limit studied. On the singular significance and interaction between factors, the factors of *Sapindus rarak* extract (A), gotu kola

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extract (B), neem seed oil (C), the interaction of *Sapindus rarak* extract and gotu kola extract (AB), interaction of *Sapindus rarak* extract and neem seed oil (AC), and interaction of gotu kola extract and neem seed oil (BC) have a p-value > 0.05 so that it does not have a significant individual effect on particle size response.

Zeta potential nanoemulgel has a value range of -9.36 ± 0.55 mV to -22.93 ± 4.31 mV. The potential zeta value is said to be stable because it meets the requirements, namely that a potential zeta value in the range of -30 mV to +30 mV will provide good stability, and a potential zeta value in the range of -60 mV to +60 mV has excellent stability. The higher the potential zeta value, the more it prevents flocculation and colloidal merger events from small to large particles. High-potential zeta will produce stable colloids. Based on previous research, the potential zeta value of contraceptive vaginal nanoemulgel as an antifungal on the market is -34 mV.[2]

The viscosity of nanoemulgel has a value range of 19,377 mPas to 19,444 mPas; this value still meets the requirements of an excellent semisolid preparation viscosity of 4,000–40,000 cPa. According to SNI 16-4399-1996, the standard viscosity value for gel preparations is 6,000–50,000 mPas. Based on previous research, viscosity testing of gotu kola extract gel (*Centella asiatica*) with a concentration of 7% obtained a viscosity value of 5,390 mPas, a concentration of 8% obtained a viscosity value of 8,060 mPas, and a concentration of 9% obtained a viscosity value of 14,250 mPas. The difference in the active ingredients used can affect the viscosity of nanoemulgel preparations.

In this study, Asiatic acid trapping efficiency results are not too close to 100% because it affects the excipient material used and the preparation's particle size. Research related to *Centella asiatica* in nanoencapsulation obtained a percentage of entrapment efficiency of less than 70%. This result was obtained because it affected the excipient surfactant gelatin material. In this study, 50% was obtained due to the influence on the concentration of dosage size using cell liposome size. Furthermore, the trapping efficiency data will be analyzed using Minitab 16.0 Response Surface Method software.

Figure 2 shows that increasing gotu kola extract with a *Sapindus rarak* extract by a concentration of 1.0% will increase the trapping efficiency response of Asiatic acid while increasing gotu kola extract with a *Sapindus rarak* extract concentration of 2.5 will decrease the trapping efficiency response of Asiatic acid. Increasing neem seed oil extract with a *Sapindus rarak* extract concentration of 1.0 will decrease the trapping efficiency response of Asiatic acid. In contrast, increasing neem seed oil extract with a *Sapindus rarak* extract concentration of 2.5 will increase the trapping efficiency response of Asiatic acid. The effect of increasing neem seed oil extract with a gotu kola extract concentration of 5.5 will tend to decrease the entrapment efficiency response of Asiatic acid. In contrast, a concentration of 7.0 will increase the trapping efficiency response of Asiatic acid. The equation for the Asiatic acid entrapment efficiency response is as follows :

$$Y = 65{,}387 - 0{,}815XA - 0{,}512\,XB + 0{,}365XC - 1{,}655XAB + 2{,}377XAC + 1{,}040XBC \ldots (2)$$

The equation model for the Asiatic acid trapper efficiency response has a p-value of 0.007 ($p < 0.05$), which is significant so that the equation model for the trapper efficiency response can be used to predict the Asiatic acid trapper efficiency response from a process condition at the concentration limit studied. All factors, namely *Sapindus rarak* extract (A), gotu kola extract (B), neem seed oil (C), interaction of *Sapindus rarak* extract and gotu kola extract (AB), interaction between gotu kola extract and neem seed oil (BC), and interaction of *Sapindus rarak* extract and neem seed oil (AC), have a p-value > 0.05. So, individually, it does not have a significant effect on the entrapment efficiency.

In this study, the results of Azadirachtin trapping efficiency were not close to 100% because it affects the excipient material used and the preparation's particle size. In studies related to azadirachtin in microcapsules, the percent of entrapment efficiency was 40–70% due to the excipient polyvinyl acetate binder of other materials. In this study, the results of neem seed oil trapping efficiency (azadirachtin) was 12.79% because the amount of polymer was higher than that of neem seed oil.[18] Furthermore, the trapping efficiency data was analyzed using Minitab 16.0 Response Surface Method software

Figure 3 shows that increasing gotu kola extract with a *Sapindus rarak* extract by a concentration of 1.0 % will reduce the trapping efficiency response of azadirachtin while increasing gotu kola extract with a *Sapindus rarak* extract concentration of 2.5 % will also decrease the trapping efficiency response of azadirachtin. Increasing neem seed oil extract with a *Sapindus rarak* extract by a concentration 1.0 % will increase the trapping efficiency response on azadirachtin. At the same time, increasing neem seed oil extract with a *Sapindus rarak* extract concentration of 2.5% will also increase the trapping efficiency response of azadirachtin. In the effect of increasing neem seed oil extract with gotu kola extract, a concentration of 5.5 % will increase the trapping efficiency response on azadirachtin, and a concentration of 7.0 will also increase the trapping efficiency response on azadirachtin. The equation for the trapping efficiency response is as follows :

$$Y = 55{,}707 + 4{,}325XA + 0{,}825XB - 10{,}790XC - 0{,}362XAB - 1{,}142XAC - 2{,}102XBC \ldots (3)$$

In Table 4, it can be seen that the significance of the equation model for the Azadirachtin trap efficiency response has a p-value of 0.037 ($p < 0.05$), which is significant so that the equation model for the trapping efficiency response can be used to predict the Azadirachtin trapping efficiency response from a process condition at the concentration limit studied.

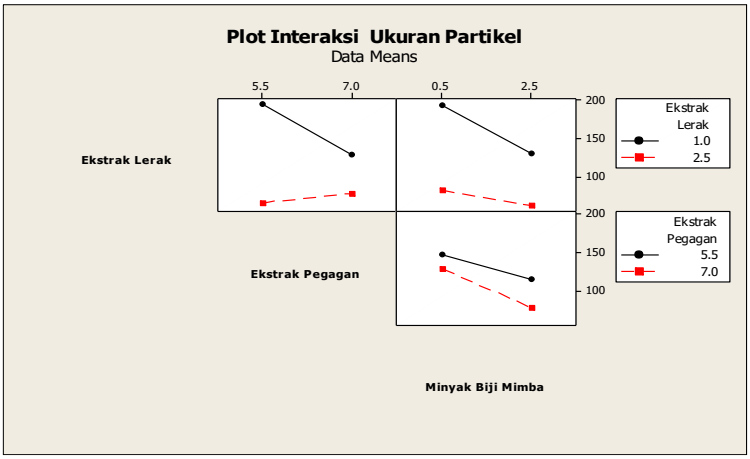

Figure 1. Particle Size Interaction Plot Graph

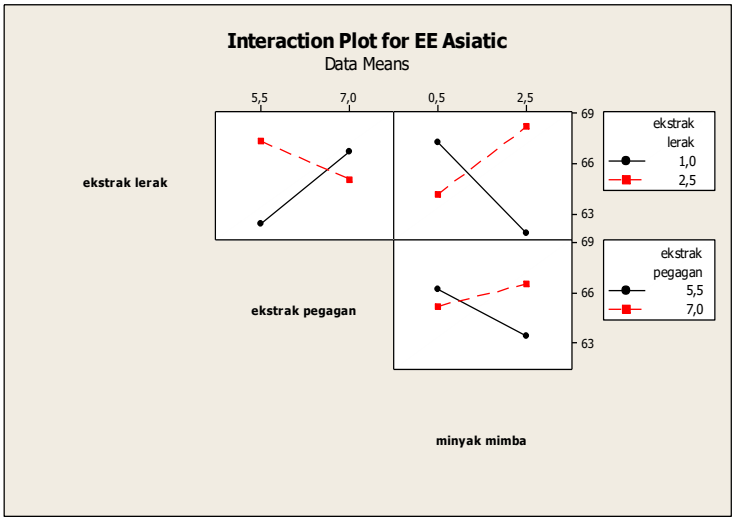

Figure 2. Effect of Asiatic acid trapping efficiency interaction on gotu kola

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Table 3: Characteristic Results of Herbal Nanoemulgel

Formula	pH ± SD	Particle Size ± SD (nm)	Polydispersity Index ± SD	Zeta Potential ± SD (mV)	Viscocity ± SD (mPas)
F I	5.19 ± 0.01	155.33 ± 1.67	0.56 ± 0.01	-18.67 ± 3.55	19.420 ± 0.01
F II	5.11 ± 0.03	153.37 ± 8.54	0.57 ± 0.07	-14.2 ± 0.62	19.433 ± 0.01
F III	5.11 ± 0.03	105.07 ± 2.31	0.93 ± 0.06	-14.73 ± 3.23	19.416 ± 0.03
F IV	5.18 ± 0.12	73.34 ± 1.54	0.96 ± 0.02	-18.56 ± 4.95	19.403 ± 0.00
F V	5.16 ± 0.06	59.66 ± 0.23	0.99 ± 0.00	-16 ± 1.73	19.377 ± 0.06
F VI	5.08 ± 0.09	234.57 ± 21.32	0.44 ± 0.05	-10.14 ± 0.20	19.444 ± 0.01
F VII	5.07 ± 0.14	52.53 ± 1.17	0.85 ± 0.02	-22.93 ± 4.31	19.423 ± 0.02
F VIII	4.89 ± 0.04	105.13 ± 2.37	0.81 ± 0.04	-9.36 ± 0.55	19.387 ± 0.01

Table 4: p-value Physicochemical Characteristics of Spermicidal Herbal Nanoemulgel

	pH	Particle Size	Polydispersity Index	Zeta Potential	Viscocity
Constant	0.005	0.066	0.019	0.096	0.000
A	0.630	0.169	0.084	0.486	0.254
B	0.367	0.470	0.443	0.929	0.589
C	0.872	0.337	0.412	0.586	0753
AB	0.859	0.355	0.198	0.737	0.477
AC	0.736	0.529	0.232	0.773	0.254
BC	0.468	0.779	0.794	0.754	0.574

All factors, namely *Sapindus rarak* extract (A), gotu kola extract (B), neem seed oil (C), interaction of *Sapindus rarak* extract and gotu kola extract (AB), interaction between gotu kola extract and neem seed oil (BC), and interaction of *Sapindus rarak* extract and neem seed oil (AC), have a p-value > 0.05. That, individually, it does not have a significant effect on the entrapment efficiency. In this study, the results of diosgenin trapping efficiency are not close to 100% because it affects the excipient material used and the preparation's particle size. While researching diosgenin on nanoparticles, a % trapping efficiency of 80.8% was obtained. This result was affected by good colloidal stability. Furthermore, the trapping efficiency data was analyzed using Minitab 16.0 Response Surface Method software.

Also, Table 4 shows that the factors of *Sapindus rarak* extract (A), gotu kola extract (B), neem seed oil (C), and the interaction of gotu kola extract and neem seed oil (BC) have the effect of increasing the efficiency response of diosgenin entrapment. Based on this figure, it can be seen that the impact of increasing gotu kola extract with *Sapindus rarak* extract by a concentration of 1.0 % will increase the trapping efficiency response in diosgenin. At the same time, the impact of increasing gotu kola extract with *Sapindus rarak* extract by a concentration of 2.5 % will also increase the trapping efficiency response in diosgenin. Increasing neem seed oil extract with a *Sapindus rarak* extract by a concentration of 1.0 % will decrease the trapping efficiency response on diosgenin. At the same time, increasing neem seed oil extract with a *Sapindus rarak* extract by a concentration of 2.5 % will also increase the trapping efficiency response on diosgenin. In the effect of increasing neem seed oil extract with gotu kola extract, a concentration of 5.5 % will increase the entrapment efficiency response in diosgenin, and a concentration of 7.0 % will also increase the trapping efficiency response in diosgenin. The equation for the entrapment efficiency response is as follows:

$$Y = 47{,}216 - 5{,}214XA - 4{,}506XB - 0{,}461XC + 1{,}754XAB + 3{,}034XAC - 0{,}619XBC \ldots (4)$$

In Table 5, it can be seen that the significance of the equation model for the Diosgenin entrapment efficiency response has a p-value of 0.014 ($p < 0.05$), which is significant so that the equation model for the entrapment efficiency response can be used to predict the Diosgenin trapping efficiency response from a process condition at the concentration limit studied. All factors, namely *Sapindus rarak* extract (A), gotu kola extract (B), neem seed oil (C), interaction of *Sapindus rarak* extract and gotu kola extract (AB), interaction between gotu kola extract and neem seed oil (BC), and interaction of *Sapindus rarak* extract and neem seed oil (AC), have a p-value > 0.05. So that, individually, it does not have a significant effect on the entrapment efficiency.

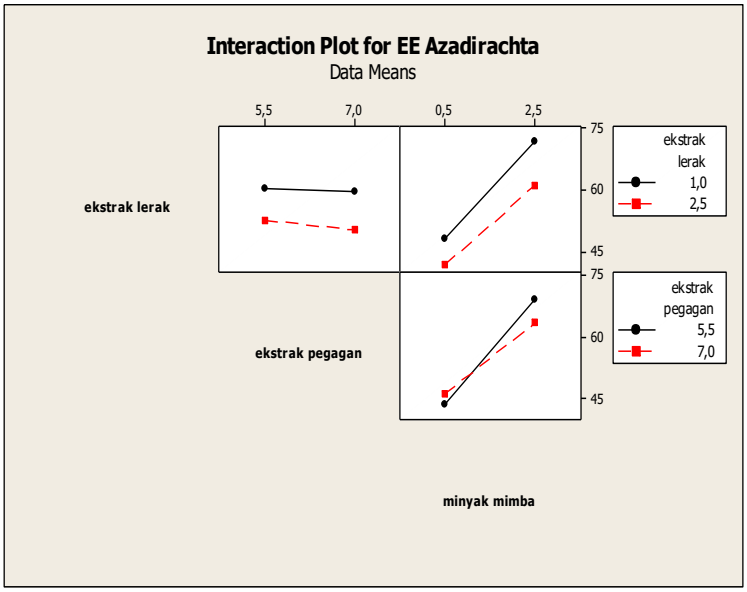

Figure 3: Effect of Azadirachtin Entrapment Efficiency Interaction on Neem Seed Oil

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Antibacterial Inhibitory Activity Assay

The antibacterial activity screening of the spermicidal herbal nanoemulgel against *Staphylococcus aureus* showed inhibition zones. The results of the inhibitory zone are shown in Table 6**.** The activity testing of the spermicidal herbal nanoemulgel combinations of *Centella asiatica* n-butanol extract, *Sapindus rarak* n-butanol extract, and neem seed oil (*Azadirachta indica*) showed activity against *Staphylococcus aureus* bacteria with the formation of inhibitory zones in eight nanoemulgel formulas. The average values of the inhibitory zones formed by the different nanoemulgel formulas, 1st -8th, were 7.10 ± 0 mm, 4.10 ± 0 mm, 5.55 ± 0.45 mm, 4.35 ± 0.25 mm, 5.55 ± 0.45 mm, 8.275 ± 0.275 mm, 8.825 ± 0.275 mm; and 4.10 ± 0 mm, respectively.[3] The positive control of Relactagel preparations containing lactic acid and glycogen has an inhibitory zone activity with an average value of 8.00 ± 0 mm and is included in the medium category (inhibitory zone 5–10 mm), while the negative control (NA) does not show anti-bacterial activity.[19] The compounds found in *C. asiatica* were known to possess antibacterial properties.[20] Based on the category of inhibitory zone activity formed, formulas 2, 4, and 8 have weak antibacterial activity (inhibitory zone <5 mm), while formulas 1, 3, 5, 6, and 7 have moderate activity (inhibitory zone 5–10 mm). Formula 7 has the best inhibitory activity against *Staphylococcus aureus* with an inhibitory zone value of 8.825 ± 0.275 mm and falls in the medium category (inhibitory zone 5–10 mm). The seventh formula has the highest concentration of *Centella asiatica* n-butanol extract, *Sapindus rarak* n-butanol extract, and neem seed oil (*Azadirachta indica*) among the eight spermicidal herbal nanoemulgel formulas. Formula 7 contain n-butanol gotu kola extract (7%), n-butanol *Sapindus rarak* extract (2.5%), and neem seed oil (*Azadirachta indica*) of 2.5%.[21] According to previous research, gotu kola ethanol extract with a concentration of 90% formed an inhibitory zone of 7.92 ± 0.42 mm, and at a concentration of 60%, formed an inhibitory zone of 7.55 ± 0.41 mm against *Staphylococcus aureus*.[22] Neem leaf ethanol extract with a concentration of 25% formed an inhibitory zone of 6.48 mm, at a concentration of 50% formed an inhibitory zone of 8.42 mm, and at a concentration of 75% formed an inhibitory zone of 12.06 mm against *Staphylococcus aureus*. *Sapindus rarak* fruit ethanol extract with a concentration of 25% formed an inhibitory zone of 0.94 mm, a concentration of 50% formed an inhibitory zone of 0.96 mm, a concentration of 75% formed an inhibitory zone of 1.02 mm, and a concentration of 100% formed an inhibitory zone of 1.03 mm against *Staphylococcus aureus*.

Antifungal Activity against Candida albicans

The spermicidal herbal nanoemulgel formulations showed inhibitory activity against *Candida albicans* with different zones of inhibition diameters. The results are presented in Table 7. The spermicidal herbal nanoemulgel combinations of *Centella asiatica* n-butanol extract, *Sapindus rarak* n-butanol extract, and neem seed oil showed antifungal activity against *Candida albicans* with the formation of inhibitory zones in 8 nanoemulgel formulas. The average values of their (1st – 8th) zones of inhibition are 7.775 ± 0.225 mm, 5.10 ± 0 mm, 5.75 ± 0.25 mm, 6.00 ± 0 mm, 7.55 ± 0 mm, 6.275 ± 0.275 mm, 9.05 ± 0 mm, 5.10 ± 0.225 mm, respectively.[1] Meanwhile, the positive control agent, Relactagel (lactic acid and glycogen), was inactive against *Candida albicans*. The negative control (Saburaud dextrose agar (SDA) media) showed no antifungal activity. Based on the category of inhibitory zone activity formed, formulas 1–8 have moderate inhibitory activity (inhibitory zone 5–10 mm). Formula 7 has the best inhibitory zone against *Candida albicans* with an inhibitory zone value of 9.05 ± 0 mm and is categorized as having a medium inhibitory activity (inhibitory zone 5–10 mm).[23] Formula 7 has the highest concentration of *Centella asiatica* n-butanol extract, *Sapindus rarak* n-butanol extract (*Sapindus rarak*), and neem seed oil (*Azadirachta indica*) among the eight spermicidal herbal nanoemulgel formulas. The content of n-butanol extracts of *Centella asiatica*, *Sapindus rarak* and neem seed oil in formula 7 is 7%, 2.5%, and 2.5%, respectively. The concentration of the extract as an active ingredient can affect the activity of the inhibitory zone; the higher the concentration of the extract in the formula, the wider the activity of the inhibitory zone formed.[24] Ethanol extract of *Centella asitica* and Neem oil showed inhibitory zones of 8.2 mm and 4 mm against *Candida* albicans, respectively. *Sapindus rarak* extract possesses compounds with possible antifungal activity. There is, however, a paucity of research information on the antifungal activity of *Sapindus rarak* extract against *Candida albicans*.[7]

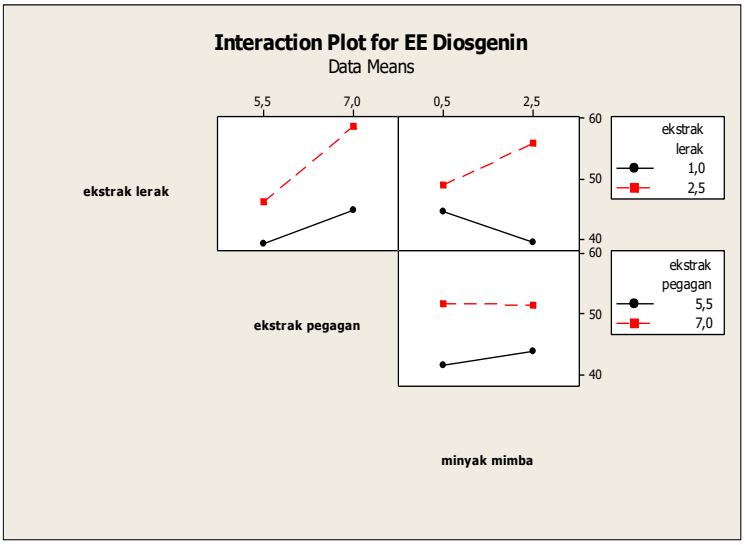

Figure 4: Effect of Azadirachtin Entrapment Efficiency Interaction on Diosgenin

Table 5: Entrapment Efficiency of Spermicidal Herbal Nanoemulgel

Formula	Entrapment Efficiency (%) *Asiatic acid*	Entrapment Efficiency (%) *Azadirachtin*	Entrapment Efficiency (%) *Diosgenin*
F I	40.61 ± 1.64	77.77 ± 0.28	36.26 ± 0.23
F II	30.79 ± 5.47	52.98 ± 0.14	46.91 ± 0.21
F III	38.76 ± 1.73	39.41 ± 0.27	56.85 ± 0.10
F IV	32.45 ± 2.08	61.08 ± 0.25	51.32 ± 0.03
F V	32.86 ± 9.72	44.06 ± 0.26	41.02 ± 0.03
F VI	34.58 ± 4.96	43.22 ± 0.15	42.24 ± 0.19
F VII	31.12 ± 5.48	60.98 ± 0.60	60.53 ± 0.04
F VIII	35.73 ± 3.65	66.16 ± 0.34	42.60 ± 0.08

The nanoemulgel pH test ranges from 4.89 ± 0.04 to 5.19 ± 0.01. This value meets the Ph

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Table 6: Nanoemulgel Inhibitory Zone Against *Staphylococcus aureus* Bacteria

Treatments	Inhibitory Zone (mm)		Average ± SD (mm)	Inhibitory Response
	1st Replication	2nd Replication		
Control + (Relactagel)	8.00	8.00	8.00 ± 0	Medium
Control – (*NA*)	0	0	0 ± 0	None
Formula 1	7.10	7.10	7.10 ± 0	Medium
Formula 2	4.10	4.10	4.10 ± 0	Weak
Formula 3	5.10	6.00	5.55 ± 0.45	Medium
Formula 4	4.10	4.60	4.35 ± 0.25	Weak
Formula 5	6.00	5.10	5.55 ± 0.45	Medium
Formula 6	8.00	8.55	8.28 ± 0.28	Medium
Formula 7	8.55	9.10	83 ± 0.28	Medium
Formula 8	4.10	4.10	4.10 ± 0	Weak

Control (+) = Relactagel preparation containing lactic acid and glycogen
Control (-) = Bacterial growth media containing nutrient agar

Table 7: Inhibitory Zone of Spermicidal Herbal Nanoemulgel Against *Candida albicans* Fungus

Treatments	Inhibitory Zone (mm)		Average ± SD (mm)	Inhibitory Response
	1st Replication	2nd Replication		
Control + (Relactagel)	0	0	0 ± 0	None
Control – (*NA*)	0	0	0 ± 0	None
Formula 1	8.00	7.55	7.78 ± 0.23	Medium
Formula 2	5.10	5.10	5.10 ± 0	Medium
Formula 3	5.50	5.50	5.75 ± 0.25	Medium
Formula 4	6.00	6.00	6.00 ± 0	Medium
Formula 5	7.55	7.55	7.55 ± 0	Medium
Formula 6	6.00	6.55	6.28 ± 0.28	Medium
Formula 7	9.05	9.05	9.05 ± 0	Medium
Formula 8	5.10	5.55	5.32 ± 0.23	Medium

Control (+) = Relactagel contains lactic acid and glycogen.
Control (-) = *Candida albicans* media contains *saburaud dextrose agar*

Conclusion

This study has reported different formulas of Nanoemulgel using different combinations and concentrations of n-butanol extracts of *Centella asiatica*, *Sapindus rarak*, and *Azadirachta indica* Seed Oil. The physical parameters of the Nanoemulgels were examined, including particle size response, zeta potential, dispersion, viscosity, and pH. The study showed no significant effects of extract concentrations on the particle size response, zeta potential, dispersion, viscosity, and pH of the nanoemulgels. The antimicrobial screening of the nanoemulgels against *Staphylococcus aureus* and *Candida albicans* showed varied inhibitory potentials, with Formula 7 exhibiting activity against the two organisms. Data obtained from this study showed that the combination of the three nanoemulgel extracts has excellent physicochemical characteristics and could be used to develop stable, effective, and safe drug delivery systems.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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