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PREPARATION OF SHALLOTS (Allium cepa L) ETHANOL EXTRACT NANOEMULSION FOR MEDICATION OF MILD BURN WOUNDS

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ABSTRACT

Shallots (Allium cepa L.) have been proven as burn wound dressings as well as topical antimicrobial agents because they can effectively heal minor burns at a concentration of 20% with a very significant decrease in the area of burns on day 1 of 5.55 cm^2 to 0.99 cm^2 on day 15 with a wound contraction percentage of 58.27%. The potential of shallot ethanol extract as a mild burn medication can be further enhanced and become more beneficial if this shallot ethanol extract is further developed as a topical nanoemulsion preparation, a nano-particle-sized material in the form of an emulsion. The purpose of this study was to determine how to formulate a topical nanoemulsion preparation of shallot ethanol extract for mild burn medication and to determine the characteristics of the topical nanoemulsion formulation. The topical nanoemulsion formulation of shallot ethanol extract for minor burns was obtained at optimum solubility conditions of olive oil-tween 80-glycerin combination with a ratio of 2:7:4 with transmittance parameters of 93.81%, total flavonoid content of 357.98 mg/36 g, polydispersity index of 0.166, and particle size of 16.63 nm, which has met the requirements as a nanoemulsion.

I. INTRODUCTION

Burns or dermatological trauma are a response of the skin or subcutaneous tissue to extreme temperatures that can be caused by direct contact such as exposure to fire (thermal), electricity (electric), chemicals (chemical), or radiation (radiation) that exceeds the tissue's ability to absorb the energy.[1] Treatment of burns has very important consequences in healing the wound. Minor burns with a certain depth, for example grade II A burns, require a fast healing time in order to suppress bacterial growth.[20] In this case, burn wound dressings or topical antimicrobial agents are usually used which function to moisturize the wound environment so as to optimize the epithelialization process and protect the skin from microorganism infection. However, the use of burn wound dressings or topical antimicrobial drugs is still constrained by their relatively expensive prices. Natural ingredients that have the potential as burn wound dressings are shallots (Allium cepa L.) which ethnobotanically have physiological activity to prevent cell damage, antibacterial, increase fibrinolytic activity so that blood flow is smoother, and as a wound medicine.[3] Shallots contain active flavonoid compounds that function as anti-inflammatories which are useful for helping to heal inflammation due to burns, bruises and inflammation of internal organs.[4] According to Yunanda and Rinanda[5], the administration of shallot extract (Allium cepa L.) topically also has a significant effect on healing cuts on the skin of mice (Mus musculus). The results of the study showed that shallot ethanol extract was effective in healing minor burns at a concentration of 20% with a very significant decrease in the area of burns on day 1 by 5.55 cm^2 to 0.99 cm^2 on day 15 with a wound contraction percentage of 58.27%. Active compounds that have the potential to help the healing process of minor burns contained in the ethanol extract of shallots (Allium cepa L.) are quercetin 3,4'-diglucoside, quercetin-3-beta-O-galactose (Hyperoside), and isorhamnetin-3-O-glucoside[6]. The potential of shallot ethanol extract as a mild burn medicine is then formulated as a topical nanoemulsion preparation to increase the activity of the extract to be more effective and efficient. How to formulate a topical nanoemulsion preparation and its physical characteristics are explained in this article.

MATERIALS AND METHODS

Materials

The materials used in the study were shallot bulbs (Allium cepa L.) obtained from Songan Village, Bangli Regency, Bali in September 2024. Plant determination was carried out at LIPI UPT. Plant Conservation Center, Eka Karya Bali Botanical Garden. The chemicals used were 96% ethanol, VCO, Chremofor RH 40, deionized water, Carbopol 940, TEA, propylene glycol and glycerin. The equipment used in the study included: glassware, analytical balance, blender, sieve, rotary vacuum evaporator, magnetic stirrer, tope bath sonicator, analytical balance, centrifuge, micro pipette, tweezers, desiccator, dropper pipette, measuring pipette, vial bottle, UV-vis Spectrophotometer, Photon Correlation Spectroscopy, electrophoretic light scattering, pH meter, and Brookfield viscometer.

Methods

Shallots Bulb Extraction (Allium cepa L.)

Shallots bulb extract was made using the wet extraction method using 96% ethanol solvent. 1 kg of shallots was blended with ethanol solvent, poured into a glass container with a lid and left for 24 hours. After 24 hours, the mixture was filtered with filter paper to obtain Shallot bulb ethanol extract. This maceration process was carried out until all secondary metabolites contained in it were completely extracted. The resulting extract was concentrated using a vacuum rotary evaporator to produce a concentrated extract. The concentrated Shallots bulb ethanol extract obtained was weighed and the flavonoid content, nanoemulsion preparation, and characteristics were determined.

Preparation, Optimization, and Characteristics of Nanoemulsion

Before making nanoemulsion, shallot extract must be able to be dissolved in the oil phase, surfactant and cosurfactant to be used, so it is necessary to conduct solubility test on shallot extract in several oil solvents, surfactants, and cosurfactants. A total of 0.5 g of extract was dissolved in 2 g of oil phase, surfactant, or cosurfactant sonicated at a temperature of 50 ± 1.0 °C for 8 hours. The sample was centrifuged at 3000 rpm for 15 minutes, then the supernatant was taken and filtered with a 0.45µm membrane. The solubility of the extract was examined on a UV spectrophotometer at a wavelength of 425 nm, replication was carried out 3 times.[7] Next, optimization of the manufacture of shallot extract nanoemulsion was carried out using the quality by design method, namely Fractional Factorial Design (FFD) and to determine the transmittance response and flavonoid levels of various formulas with a combination of oil, surfactants, and cosurfactants.

Weighed 1 g of oil extract, surfactants, and cosurfactants so that the total amount was adjusted to 10 g. Sonication for 1 hour at a temperature of 50°C followed by stirring using an overhead stirrer at 1000 rpm for 5 minutes. Add 25 mL of water, then the mixture was stirred using an overhead stirrer at 1000 rpm for 5 minutes, sonication was carried out for 1 hour at a temperature of 50°C. Finally, stir again using an overhead stirrer at 1000 rpm for 5 minutes. Determination of the characteristics of the physical properties of the optimal nanoemulsion formula as follows:

a. Clarity Test/Transmittance Determination

Observation of the clarity of the formed nanoemulsion can be done by measuring the UV-Vis spectrophotometer using the % transmittance parameter at a wavelength of 650 nm with aqua deion as a blank. A good nanoemulsion will have a clear visual with a percent transmittance value of 90-100%.

b. Particle Size Measurement

The nanoemulsion droplet size and polydispersity index were determined using Photon Correlation Spectroscopy. A total of 1 g of extract nanoemulsion was dispersed in 5 mL of aqua deion and measured. Droplet size testing was carried out to see whether the resulting nanoemulsion preparation had a droplet size that met the nanoemulsion droplet size criteria, namely <100 nm.[7]

RESULTS AND DISCUSSION

Shallot Bulb Extraction Results (Allium cepa L.)

The results of shallot bulb extraction were obtained as much as 280.60 g (yield 9.354%). To determine the flavonoid content in the shallot extract sample, the flavonoid content in the extract was determined, which obtained the following results:

Table 1: Results of Total Flavonoid Level Test of Shallots Extract.

Sample	Replication	Flavonoid Content in Shallot bulb Extract (%)	Average (mg)	Flavonoid Content in Shallot bulb Extract (%)
test solution extract	1	1.837	1.84	0.18%
	2	1.838		
	3	1.839		

Nanoemulsion Preparation, Optimization, and Characteristics

Nanoemulsion is a transparent or translucent emulsion system and is an oil-water dispersion stabilized by surfactants that have a droplet size of 50-500 nm. Nanoemulsions have several advantages, including having a larger surface area and being energy-free, non-toxic, and non-irritating, so they can be easily applied through the skin or mucous membranes. Nanoemulsions can also increase absorption, increase drug penetration, and help solubilize hydrophobic active substances.[8]

Nanoemulsion preparations generally have components such as oil, surfactants, and cosurfactants that should not irritate and be sensitive to the skin in particular. Oil is an important component in nanoemulsion formulations because it can dissolve lipophilic active ingredients. Non-ionic surfactants are generally used because they have low toxicity compared to ionic surfactants. In most cases, the use of surfactants alone is not sufficient to reduce the interfacial tension between oil and water, so cosurfactants are needed to help reduce the interfacial tension. The addition of cosurfactants can not only reduce the oil-water interfacial tension, but can also increase the fluidity at the interface so that it can increase the entropy of the system. Cosurfactants can also increase the mobility of the hydrocarbon tail so that oil penetration in the tail becomes greater.[9] To make nanoemulsions, shallot extract must be able to be dissolved in the oil phase, surfactant and cosurfactant to be used. Therefore, it is necessary to test the solubility of shallot extract in several oil solvents, surfactants and cosurfactants. The solubility of the extract was determined in various oils such as oleic acid, coconut oil and olive oil, in surfactants such as Tween 20, Tween 80 and cremophor RH 40, and co-surfactants such as PEG 400, propylene glycol and glycerin. The results of the solubility test of shallot extract in various solvents on total flavonoid levels are as follows:

Table 2: Results of the solubility test of shallots extract on total flavonoid content.

No	Solvent Name	Level Total Flavonoids (μg/g)
Oil		
1	Oleic Acid	46.808
2	Coconut Oil	43.579
3	Olive Oil	49.990
Surfactant		
1	Tween 20	57.872
2	Tween 80	186.897
3	Cremophor RH40	56.282
Cosurfactant		
1	PEG 400	82.949
2	Propylene Glycol	686.000
3	Glycerin	587.256

Based on Table 2 above, it is known that the oils that can be used in making nanoemulsions are oleic acid and olive oil, while the surfactants are Tween 80 and Tween 20 so the cosurfactants that can be used are glycerin and propylene glycol.

Nanoemulsion formula optimization was carried out using the fractional factorial design (FFD) method with a choice of combinations of oil materials (oleic acid and olive oil), surfactants (Tween 20 and Tween 80) and cosurfactants (glycerin and propylene glycol).

Table 3: Design of experimental FFD 26-2.

Level	Factors					
	Numerical factors			Categorical factors		
	1	2	3	4	5	6
Lower level (-1)	1	4	1	Oleic Acid	Tween 20	Glycerin
Upper level (+1)	2	7	4	Olive Oil	Tween 80	Propylene Glycol

1, oil proportion; 2, surfactant proportion; 3, cosurfactan proportion; 4, oil type; 5, surfactan type; 6, co-surfactant type.

The following are the transmittance responses and flavonoid levels of various formulas with a combination of oil, surfactants, and cosurfactants shown in Table 4 below.

Table 4: Transmittance responses and flavonoid levels in various formulas with a combination of oil, surfactants, and cosurfactants.

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Response 1	Response 2
Run	A: Minyak	B: Surfactant	C: Cosurfactant	D: Types of Oil	E: Types of Surfactants	F: Types of Cosurfactants	Transmitance (% T)	Drug Content (Total Flav) mg/36 g
1	2	7	1	Olive Oil	Tween 20	Propylene Glycol	7.7	62.769
2	2	4	4	Oleic Acid	Tween 20	Glycerin	32.7	114.795
3	1	4	4	Oleic Acid	Tween 80	Glycerin	77.25	83.538
4	2	7	4	Olive Oil	Tween 80	Glycerin	96.48	540.333
5	1	7	4	Olive	Tween 20	Glycerin	28.09	125.308

				Oil				
6	2	7	1	Oleic Acid	Tween 20	Glycerin	75.82	90
7	1	7	1	Oleic Acid	Tween 80	Glycerin	94.36	86.359
8	2	4	4	Olive Oil	Tween 20	Propylene Glycol	6.16	294.59
9	1	4	1	Olive Oil	Tween 20	Glycerin	1.01	89.641
10	2	7	4	Oleic Acid	Tween 80	Propylene Glycol	74.23	82.513
11	1	7	4	Oleic Acid	Tween 20	Propylene Glycol	80.09	64.103
12	1	7	1	Olive Oil	Tween 80	Propylene Glycol	87.29	32.769
13	2	4	1	Olive Oil	Tween 80	Glycerin	87.1	67.846
14	1	4	1	Oleic Acid	Tween 20	Propylene Glycol	78.28	60.359
15	1	4	4	Olive Oil	Tween 80	Propylene Glycol	3.49	585.462
16	2	4	1	Oleic Acid	Tween 80	Propylene Glycol	10.72	45.154

The following is documentation of the resulting nanoemulsion:

Figure 1: Nanoemulsion in various formulas.

Optimization Results using FFD

Based on the preparation results carried out in accordance with the design made, a shallot bulb extract nanoemulsion was obtained with a transmittance response between 0.13% to 98.49% and a drug content between 20.01 mg to 37.58 mg.

Table 5: Analysis of Variance (ANOVA) of shallot bulb extract nanoemulsion.

Respon	*F value*	*P value*	*R2*
% Transmitance	20.73	0.0002 (significant)	0.9478
% Drug Content	26.14	0.0007 (significant)	0.7447

Determination of Optimum Formula of Shallots Extract Nanoemulsion

The results of determining the optimum formula for shallots extract nanoemulsion are presented in Table 6 below:

Table 6: Optimum Formula for Red Onion Extract Nanoemulsion.

Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Response 1	Response 2	Desirability
A Oil	B Surfactant	C Cosurfactant	D: Types of Oil	E: Types of Surfactants	F: Types of Cosurfactants	Transmitance (% T)	Drug Content (Total Flav) mg/36 g	
2	7	4	Olive Oil	Tween 80	Glycerin	93.909	365.650	0.885

The calculation results presented in Table 6 show that the optimal nanoemulsion is a combination of olive oil-tween 80-glycerin with a ratio of 2:7:4. The formula is calculated to be targeted to produce a transmittance of 93.909% and a flavonoid content of 365.650 mg/36 g.

Determination of Transmittance, Total Flavonoid Content, Particle Size, and Polydispersity Index of Optimal Formula

An optimal formula was created with a combination of olive oil: tween 80: glycerin with a ratio of 2: 7: 4. Then the transmittance, total flavonoid content, particle size, and polydispersity index of the optimal formula were determined and the following results were obtained:

Table 7: Results of measurements of Transmittance, Total Flavonoid Content, Particle Size, and Polydispersity Index of the Optimal Nanoemulsion Formula.

No.	Test Type	Observation result	Average	Condition	Results
1.	Transmitance (%)	92.550	93.81	Maximum	Qualify
		93.335			
		95.557			
2.	Drug Content (Total Flav) mg/36 g	359.750	357.98	Maximum	Qualify
		358.500			
		355.690			
3.	Polydispersity Index	0.148	0.166	< 0.500	Qualify
		0.119			
		0.230			
4.	Particle size (nm)	16.9 nm	16.63	10 – 200 nm	Qualify
		16.7 nm			
		16.3 nm			
		0.1 mV			
		0.1 mV			

The data in Table 7 shows the optimal formula with a combination of olive oil: tween 80: glycerin with a ratio of 2: 7: 4 produces a visually clear formula with a transmittance value of > 90%. This indicates the formation of a nanoemulsion that is able to produce small particle sizes. The percentage of transmittance values approaching 100% indicates that the particle size of the nanoemulsion preparation is very small so that it can pass through the light beam and shows high transmittance measurements.[10] The transmittance value increases with decreasing particle size. This is due to the multimodal distribution of particles, namely larger particle sizes will scatter light more strongly. Surfactant affinity also greatly affects the particle size and hydrophilic or hydrophobic phase of the nanoemulsion preparation, the type of surfactant used is an important factor in making nanoemulsion preparations. Various types of surfactants have the ability to carry out different emulsification processes due to their molecular geometry.[11] The drug content is also greater than the extract before being made into a nanoemulsion. This shows that there is a correlation between particle size and its active side. The smaller the particle size, the larger the surface area and the more active sides so that its interaction with the emulsifying solvent is greater and the active compound extracted is also greater. The polydispersity index is a value that describes the distribution of particle size or the uniformity of the particle size of the preparation. The polydispersity index value that is getting closer to 0 indicates that there is no aggregation and the particle size is uniform. The polydispersity index value of the nanoemulsion preparation that can be categorized as monodispersity and homogeneous is <0.3. Based on the results of the polydispersity index, it can be seen that the optimal formula can be categorized as monodispersity because it has a polydispersity index of <0.3.[12] Stirring speed is a factor that affects the polydispersity index value of the nanoemulsion preparation. Stirring too fast causes the particle size to become large and the preparation to be cloudy, while stirring too slow causes the preparation to be inhomogeneous. Homogeneous preparations will have a smaller polydispersity index value, while heterogeneous preparations will have a larger polydispersity index value. In this study, it can be concluded that the stirring speed used was appropriate because it was able to produce preparations that were physically homogeneous and had good polydispersity index values.[13]

Particle size is an important parameter in the manufacture of nanoemulsion preparations, considering that one of its characteristics is having a particle size of 1–100 nm. Particle measurements were carried out to determine the particle size of the nanoemulsion preparation using a PSA instrument. In addition to particle size, other information obtained during PSA testing is the polydispersity index value.

Based on Table 7, it can be seen that the optimal formula has a particle size in the range of 16.63 nm. This shows that the preparation made meets the standards for making good nanoemulsions, where the nanoemulsion preparation has a particle size <100 nm.[14] The particle size in nanoemulsion preparations can affect the rate of drug release and absorption. The smaller the particles formed, the faster the drug is absorbed by the body, thereby accelerating the desired therapeutic effect.[15] The choice of homogenization method affects the particle size of the nanoemulsion preparation. The formulation of the nanoemulsion preparation of shallot ethanol extract uses a sonicator, a high-energy method in an effort to reduce the particle size of the nanoemulsion preparation. The working principle of the sonicator is to convert ultrasonic waves into vibrations by utilizing electrical energy so that the vibrations will break the particles into smaller sizes.[16] In addition to the homogenization method used, the surfactant component also affects the particle size of the nanoemulsion preparation. Tween 80 is able to prevent particle size enlargement by reducing the amount of free energy between surfaces and forming a mechanical barrier. This barrier functions to prevent aggregation which can cause particle size to increase.[17] Based on the data in Table 7, it can be concluded that the optimal formula of shallot oil nanoemulsion with a combination of olive oil: tween 80: glycerin with a ratio of 2: 7: 4 meets the requirements as a nanoemulsion and can thus be further developed into a nanoemulgel.

Figure 2: Formula nanoemulsi optimal (olive oil: tween 80 : gliserin = 2 :7 : 4)

CONCLUSION

The optimum condition of the topical nanoemulsion formulation of shallot ethanol extract for minor burns is the combination of olive oil-tween 80-glycerin with a ratio of 2:7:4 with transmittance parameters of 93.81%, total flavonoid content of 357.98 mg/36 g, polydispersity index of 0.166, and particle size of 16.63 nm, which has met the requirements as a nanoemulsion.

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