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Formulation, Characterization, and Optimization of Snakehead Fish (*Ophiocephalus Striatus*) Powder Nanoemulgel

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ABSTRACT

Snakehead fish powder was formulated into nanoemulgel utilizing the best comparison of surfactant, co-surfactant and oil. The aims of this study was to determine physical stability and characterization of snakehead fish powder nanoemulgel by spontaneous emulsification method. PEG 400 was added into Tween 80 and olive oil then water, containing 0.1% of snakehead fish powder, was added drop by drop until becoming a transparent solution by sonication. Nanoemulsion was added with 1.5% HPMC gel and mixed until nanoemulgel form. It was characterized by UV-Vis Spectrophotometry and DLS. The results of this research showed that snakehead fish nanoemulgel produced clear, stable, and transparent formula having the transmittance value of 98.825%. The characterization results described nanoemulgel had the average of particle size, PDI, and zeta potential were around 2.9 nm, 0.589 and -60.72 mV respectively. This means that nanoemulgel was stable having a uniform particle size, pH 5, and the viscosity value of 210 cP. The results of the evaluation of stability test showed a good level of stability with the viscosity and pH by one way ANOVA analysis which did not change significantly.

Keywords: Nanoemulgel, powder, particle size analyzer, snakehead fish.

INTRODUCTION

In Indonesia, one of freshwater fish species having main function for human health is snakehead fish (*Ophiocephalus striatus*). It contains high protein such as albumin, amino acids and unsaturated fatty acids which are often used by people to accelerate wound healing with formation of new tissues.[1] Besides that, albumin in snakehead fish was utilized to accelerate burn wound healing, increase the amount of blood protein, improve fracture and prevent lung infection.[2] According to Tungadi (2011), snakehead fish powder had been formulated into macro emulsion cream for accelerating wound healing of post-operation in vitro.[3] In general, this cream was physically unstable so that the emulsion system was easily broken by adding energy of oil, water phase and storage temperature. This problem can be solved by reducing particle size of active compounds and stabilizer use. The reduction of particle size or droplet oil-water can be done by making nanoemulsion with the comparison of surfactant, co-surfactant, and oil appropriately and the addition of gelling agent such as HPMC as called nanoemulgel.[4]

The snakehead fish powder can be formulated into emulgel dosage form because the contents of the fish consists of polar (water soluble protein) and nonpolar (fatty acid soluble oil) parts. Nanoemulgel can be used as transdermal drug delivery system which has to pay attention to penetration of drug active compounds because it has to be able to pass through the skin barrier i.e. stratum corneum. Meanwhile, Tungadi (2016) stated that snakehead fish cream (negative control) was difficult to penetrate stratum corneum using rabbits in vivo which could be seen open wound longer recovery than using penetrant enhancer such as propylene glycol (treatment group). This means that

propylene glycol can accelerate the diffusion rate of albumin into stratum corneum which the amount of albumin around 50% compared to without penetrant enhancer about 5-7%. Therefore, snakehead fish powder was formulated into nanoemulgel.[5]

Nanoemulgel consists of nanoemulsion and gel which can increase the diffusion rate of active compounds because it reduces the particle size or droplets from oil and water phase in the emulsion system. Smaller particle size can enhance extensive contact with the membrane cell particles and facilitate carrier particles for penetration into membrane cells. So, the amount of drug active compounds was easy to penetrate systemic circulation which will increase bioavailability of active compounds. This means that it does not need a penetrant enhancer to accelerate the diffusion rate of active compounds into membrane cells.

The formulation of snakehead fish powder used a spontaneous emulsification method with comparison of surfactant, co-surfactant and oil appropriately such as tween 80, PEG 400 and olive oil. The characterization of snakehead fish powder nanoemulsion had important roles in showing stability measurements such as particle size, zeta potential, and poly-dispersion index by particle size analyzer. This can prove that snakehead fish powder, formulated into nanoemulsion utilizing spontaneous emulsification method, can reduce particle size of snakehead fish powder.

MATERIALS AND METHODS

Materials: Snakehead fish powder of pharmaceutical grade was gained by PT. Royal Medical Pharmaceutical, Indonesia, and was certified containing protein 85.6%, albumin 30.2%, omega-3 2.03%, omega-6 2.11% and omega-9 0.92% and polyunsaturated total 5.1% respectively. The gelling agent, HPMC 22.000 1.5% b/v, was purchased from PT. Brataco Chemical. Basis of Nano-emulsion consisted of Tween 80 30% b/v (surfactant), PEG 400 25% b/v (Co-surfactant), and Olive oil 5% b/v (oil). All of them were bought from PT. Intraco Chemical. DMDM Hydantoin 0.1% b/v and BHT 0.1% b/v were purchased from PT. Sentana Chemical. The UV-Vis Spectrophotometry was from Perkin Elmer (USA). The Delsa^{TM} Nano having particle size of 1 nm – 700 nm (UK). In addition, a pH meter (Systronics model EQMK), a sonicator (Specta Lab, model UC 40) and a hot air oven (Memmert) were utilized in this study.

Optimization of gel basis: HPMC was made in different concentrations i.e.1.5% b/v, 2.0% b/v, and 2.5% b/v respectively. Each concentration of HPMC was weighed according to concentrations of each formula. After that, HPMC was dispersed into warm water (70^{o}C) and allowed to stand for 15 minutes then stirred 500 rpm for 3 minutes to form a clear gel with appropriate viscosity.

Optimization of nanoemulsion basis: The optimizations of Nano-emulsion basis were made in different comparisons of surfactant, co-surfactant and oil. There were 6 formulas with the comparison of different concentrations between surfactant (tween 80), co-surfactant (PEG 400) and oil (olive oil) such as F1 (4:2:1), F2 (4:3:1), F3 (5:3:1), F4 (6:3:1), F5 (7:3:1), and F6 (7:4:1). Tween 80 and PEG 400 were mixed together utilizing magnetic stirrer for 30 minutes 250 rpm (the first mixture). After that, olive oil was added to the first mixture while stirring and adding water drop by drop containing snakehead fish powder 0.1% b/v then done sonication for 10 minutes. The same procedure was made for all formulas with different comparisons of tween 80, PEG 400, and olive oil.

Formulation of snakehead fish powder nanoemulgel: After optimization of nanoemulsion basis, getting the best formula of 6 formulas was continued by formulating snakehead fish powder using spontaneous emulsification method. The best formula had small particle size, transparent and very clear which was formulated into nanoemulgel containing snakehead fish powder. The best optimization of nanoemulsion was added into gel basis with the best concentration of HPMC optimization then stirred until getting homogeneous nanoemulgel. This Formula, giving transparent emulgel, was continued by transmittance measurement using UV-Vis Spectrophotometry.

CHARACTERIZATION AND EVALUATION

The particle size measurement of snakehead fish powder nanoemulgel: Particle size, potential zeta, and poly-dispersion index by particle size analyzer (PSA) were measured from snakehead fish powder by putting nanoemulgel in the cuvette of PSA. Then this was measured by zeta sizer which is shown in graph.[6]

The evaluation of snakehead fish powder nanoemulgel: The evaluation of snakehead fish powder nanoemulgel involved organoleptic, pH, viscosity, and stability tests including centrifugation and freeze-thaw methods.[7,8] All data were statistically analyzed by utilizing one-way Anova.

RESULTS AND DISCUSSION

Optimization of gel basis: The results of gel basis optimization in different concentrations i.e. HPMC 1.5% b/v, 2% b/v, and 2.5% b/v respectively show that HPMC 1.5% b/v gave the best result with clear

physical appearance and appropriate viscosity (**Table 1.**). HPMC 1.5% b/v as gelling agent has low viscosity; so, that it is easy for snakehead fish powder to penetrate into skin cell membrane. Otherwise, the other concentrations have high viscosity which can affect snakehead fish powder penetration into skin cell membrane. HPMC has the ability to spread better than carbopol, methylcellulose, and sodium alginate and is also easy to apply to skin.[9,10] Besides that, the advantages of HPMC are neutral, stabile viscosity, resistant to microbial growth, clear gel and strong film on the dry skin.[11]

Optimization of nanoemulsion basis: The optimization of nanoemulsion basis indicates formula 6 with the comparison of olive oil, Tween 80 and PEG 400 (1:7:4). This has the best performance including viscosity, clarity, and stability (**Table 2.**). Formula 6 was physically stable utilizing centrifugation method (3800 rpm; 5 hours). The formula did not segregate. Otherwise, formulas 1 to 5 gave cloudy appearance and showed segregation after centrifugation. Tween 80 as nonionic surfactant has high hydrophilic and lipophilic balance (15) so that it can be stable in an emulsion system with oil in water.[12] This surfactant has pivotal roles in nanoemulsion basis because it has a large surface area for reducing interfacial and surface tension causing the surfactant to be absorbed on interface phase. Regarding this, it can decrease the surface free energy by ruining globule and resulting small globule.[13] The most surfactants are not able to reduce interfacial tension in emulsion so that it needs to add co-surfactant (PEG 400) which can increase the solubility of nonpolar groups.[14] Besides that, it can intensify flexibility of surfactant film and fluidity of emulsion phase.[15]

Formulation of snakehead fish powder nanoemulgel: The particle size of snakehead fish powder, containing albumin, protein, and amino acids, used in formulation, was 30 μm. It means that the particle size of powder has important roles in accelerating drug diffusion to penetrate into membrane cell. Therefore, the snakehead fish powder was formulated into nanoemulgel to reduce particle size of powder using a spontaneous emulsification method. Due to small particle size from emulsion, the powder can accelerate active compounds such as albumin, protein, and amino acids to penetrate membrane cells. It causes the increase of the contact area between the carrier particles and the membrane cell.[16] Regarding this, the carrier particles are easy to release active compounds into systemic circulation system causing the increase of bioavailability of snakehead fish nanoemulgel and the acceleration of wound healing processes.

CHARACTERIZATION AND EVALUATION

The particle size measurement of snakehead fish powder nanoemulgel: The characterization of snakehead fish nanoemulgel utilized UV-Vis Spectrophotometer and particle size analyzer. The best formula of snakehead fish nanoemulgel, transparent emulgel, measured transmittance on the wavelength 650 nm and resulted in 98.82%. After that, it was continued by particle size analyzer to know particle size, zeta potential and poly-dispersion index of snakehead fish nanoemulgel. The results of characterization show that the average of particle size of snakehead fish nanoemulgel was 2.9 nm of three replications (3.5, 3.3, and 1.9 nm). It proves that snakehead fish nanoemulgel meets the criteria of nanostructures which are the particle size range of nanoemulsion 1 – 100 nm or 2 – 500 nm.[17] Meanwhile, zeta potential value of snakehead fish nanoemulgel was -60.72 mV. It means that snakehead fish nanoemulgel has a good degree of stability because zeta potential value describes the potential of the particles charge. Nano particles with the value of potential zeta above or below ±30 mV show that colloidal system is physically stable; so that the magnitude of the particle charge can prevent particle aggregation based on electrostatic repulsion.[18] Besides that, poly-dispersion index of snakehead fish nanoemulgel give good results of three replications i.e. 0.988, 0.638, and 0.141 respectively (**Table 3.**). The average of poly-dispersion index was 0.589. This means that snakehead fish nanoemulgel has uniform particle size and homogeneous dispersion due to the value of poly-dispersion range between 0 and 1.

The evaluation of snakehead fish powder nanoemulgel: Based on freeze thaw method, snakehead fish nanoemulgel presented good results which were physically stable at extreme temperatures (4ºC and 40ºC) for 7 cycles (28 days). Regarding this, observation results showed that the viscosity value of snakehead fish nanoemulgel was around 210 cP and the pH value about 4.5 – 5.0 in different cycles. All data are presented in **Table 4**. Meanwhile, the statistical analysis of freeze thaw method particularly pH (**Table 5.**) and viscosity (**Table 6.**) data showed that P value is greater than 1 which mean there is no real difference between pH and viscosity of the stability test. It means that there were no significant changes (one-way Anova) involving organoleptic tests i.e. clear, homogeneous, and slightly viscous. Meanwhile, centrifugation methods described snakehead fish nanoemulgel was stable which marked no segregation between gel and emulsion for 5 hours, 3800 rpm. This means that formulas of snakehead fish nanoemulgel can be saved for 2 years.[15]

CONCLUSION

It can be concluded that all of evaluation results and stability tests showed snakehead fish nanoemulgel has good stability including the characterization of snakehead fish nanoemulgel which obtained data such as particle size average 2.9 nm, zeta potential -60.72 mV and poly-dispersion index 0.589.

Conflicts of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Table 1. The Optimization Result of Gel Basis

Materials	**F1 (%)**	**F2 (%)**	**F3 (%)**
HPMC	1.5	2	2.5
Distilled Water	100	100	100
Observation	Clear	Clear	Clear
pH	5.0	5.0	5.0

Table 2. The Optimization Result of Nanoemulsion Basis (without snakehead fish powder)

	Formula % b/v					
Materials	F1 (1:2:4)	F2 (1:3:4)	F3 (1:3:5)	F4 (1:3:6)	F5 (1:3:7)	F6 (1:4:7)
Olive oil	5	5	5	5	5	5
Tween 80	17	19.5	22	24.5	27.5	30
PEG 400	13	15.5	18	20.5	22.5	25
Distilled water	100	100	100	100	100	100
Observation	cloudy	cloudy	cloudy	cloudy	cloudy	clear
pH	7.0	7.0	7.0	7.0	7.0	7.0

Table 3. The Characterisation Result of Snakehead Fish Nanoemulgel

Sample (F6)	**Particle Size (nm) 3 replications**	**Poly-dispersion Index**	**Potential Zeta (mV)**
Snakehead fish	3.5 ± 0.9	0.988	
Nanoemulgel	3.3 ± 0.9	0.638	-60.72
	1.9 ± 0.5	0.141	

Table 4. The Evaluation Results of Freeze-Thaw Method (basis + snakehead fish powder)

Treatment (F6)	**Temperature (°C)**	**Viscosity (cP)**	**pH**
Cycle 1	40 4	210	5.0
Cycle 2	40 4	210	5.0
Cycle 3	40 4	210	5.0
Cycle 4	40 4	209	4.9
Cycle 5	40 4	208	4.9
Cycle 6	40 4	208	4.9
Cycle 7	40 4	207	4.8

Table 5. One-Way Anova for pH Of Snakehead Fish Powder Nanoemulgel

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
pH of snakehead fish powder nanoemulgel on stability test of freeze thaw method.	Between Groups	.000	1	.000	.000	1.000
	Within Groups	.027	12	.002		
	Total	.027	13			
The treatment tests	Between Groups	.000	1	.000	.000	1.000
	Within Groups	56.000	12	4.667		
	Total	56.000	13			

Table 6. One-Way Anova for Viscosity Of Snakehead Head Powder Nanoemulgel

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Viscosity of snakehead fish powder nanoemulgel on stability test of freeze thaw method.	Between Groups	.000	1	.000	.000	1.000
	Within Groups	17.714	12	1.476		
	Total	17.714	13			
The treatment tests	Between Groups	.000	1	.000	.000	1.000
	Within Groups	56.000	12	4.667		
	Total	56.000	13			

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