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Evaluation Of Physical Stability And Antibacterial Properties Of Snakehead Fish *(Ophiocephalus Striatus)* Cream Containing Immunoglobulin

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Abstract : Snakehead fish (*Ophiocephalus striatus*) powder contains immunoglobulins as well as possessing antibacterial properties. The aim of this study was to investigate physical stability of creams containing snakehead fish powder and *the in vitro* antibacterial activity against selected human pathogens.The snakehead fish blended and squeezed to express a liquid with water and oil phases. Water phase fraction was driedby atomizer as powder then made into o/w cream by using nonionic emulgents (Tween and Span 60) of 2%, 3% and 4%. The cream was made containing 2% snakehead fish powder. Physical properties of creams such as organoleptic, creaming, viscosity, phase inversion, and pH test were evaluated. Creams, which were stable physically, were subjected to preliminary screening for antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa.* The agar diffusion method was used to determine the inhibition zones of snakehead fish cream 2% in mm (3 replications).Organoleptic observations showed no change in color and odor for all of cream formulations before and after stress conditions. Creaming test showed that all of the formulaswere stable or showed no creaming after stress conditions. Statistical analysis using Completely Randomized Design (CRD) showed that the concentrations of nonionic emulgents gave a real influence on the cream viscosity before and after accelerated storage conditions. Phase inversion tests stated that all of the formulas did not show emulsion type change. Finally, pH test indicated that all of the formulas had pH 5 – 6, the same as pH of skin. Meanwhile, there was no inhibitory zone forthe creams against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, but the snakehead fish cream showeda radial zone of inhibition against *Bacillus subtilis*for all three replicates (23, 20, and 22 mm).All cream formulations with different emulgent concentrations can be categorized as physically stable and snakehead fish cream of 2% had antibacterial activity against *Bacillus subtilis.*
Keywords : antibacterial properties, cream, emulgent, non-ionic, snakehead fish.

Introduction

Nowdays, modern drugs particularly antibiotic has experienced a lot of resistance against pathogen bacteria in the human body. Therefore, it needs to explore new active compounds which derived from natural products such as animals or plants and have function as new antimicrobial compounds[1].

Snakehead fish (*Ophiocephalus striatus*) is one of fresh water fish which can grow up till 1 metre, big head such as snake and bread in river, rice field, and swamp[2]. This fish contain high protein particularly protein total (85.60%), albumin (30.20%), lipids (5.1%), mineral and amino acids. Besides that, snakehead fish

powders contain immunoglobulin (Ig+) 2.11 IU/g as antibacterial component[3]. Ig+ has important role in boosting immune system in the human body. Regarding this, snakehead fish powder is often utilized by people to heal open wounds in a way sow the powder on the wound skin surface so that the wound can quickly dry. On the other hand, the usage of snakehead powder on the wound directly can cause uncomfortable feeling for some people. Because this can give infection on the skin by contamination of bacteria which can exposure directly in the air. Therefore, snakehead fish powder is more elegant designed into cream dosage form.

The application of powder drug on the skin can be achieved by semisolid dosage forms such as ointment, cream, pasta and gel. In this research, snakehead fish powder was formulated into cream containing two active compounds such as polar compound (albumin) and nonpolar compound (omega-3 fatty acids). According to Taslim et.al.the giving of snakehead fish extract of 100 mL for 5 days escalated the level of albumin and protein total of patients. Meanwhile, Filix stated that the use of snakehead fish extract against wound healing on oral cavity required healing time for 10 days. In addition, Tungadi explained that 2% of snakehead fish cream accelerated wound healing process on rabbit's skin histopathologically[4].

Based on the previous results of snakehead fish research were done formulation development by testing stability of snakehead fish cream and antimicrobial activity. For stabilizing an emulsion is needed an emulsifier which can decrease interfacial tension between oil and water phase. One of emulsifiers which can be utilized for topical use i.e. polioksietilensorbitan monostearate and sorbitan monostearate which often utilized into cream or cosmetic products[5,6]. They showed low toxicity and irritation which also have homogenous degree with other materials[7]. The main thing in determining emulsion stabilities is shelf life prediction from system under normal storage condition which can be accepted on room temperature[8].

Regarding this, snakehead fish cream 2%, containing different concentrations of emulsifiers, was evaluated by some instruments to know physic stability and antibacterial activity of cream utilizing *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* Therefore, the aims of this research to determine concentrations of non-ionic emulsifier which can produce stable snakehead fish cream physically and high inhibition zone in vitro.

Materials and Methods

Snakehead fish powder was gained from PT. Royal Medica Pharmaceutical using atomizer (-40^{o}C; 1 atm). Emulsifiers,tween 60 and span 60 were bought from PT Intraco. Gram positive bacterium such as *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (ATCC 6633). Gram negative bacterium such as *Pseudomonas aeruginosa* (ATCC 27853).Agar nutrient medium.All bacterium and medium were gained from Pharmacy Microbiology Laboratory, UNHAS. Brookfield ViscometerDV-E series. Petri dishes and paper disc were gained from microbiology laboratory, UNG.

Preparation of Snakehead fish dry extract

Snakehead fish were cleaned and cut like dice form. After that, they were entered into atomizerextraction tool to get dry extract of snakehead fish[3].

Preparation of snakehead fish cream

The snakehead fish dry extract of 2% was designed into 3 formulas utilizing polioksietilen sorbitan monostearate (Tween 60) and sorbitan monostearate (Span 60) with different concentrations i.e. 2%, 3%, and 4% respectively. Cream was made by melting oil and water phase on waterbath 70^{o}C then mixed oil phase into water phase while stirred using ultraturax 3 minutes; 3800 rpm. Before mixing oil into water phase, snakehead fish dry extract had been dissolved into water phase. Snakehead fish cream 2% was made into o/w type of emulsion.

Emulsion type determinations

a. Dilution method: creams were put into vial then added water sufficiently and observed color change.

b. Dispersion dye method: creams were put into vial then dropped with blue methylene and observed changes.

c. Electrical conductivity method: creams were put intoglass beaker which connected with circuit electric current and observed the light bulbs.

Evaluations of physical stability of snakehead fish Cream

a. Organoleptic observations: Cream was done observation including change in odor, color, and physical appearance. This observations were done before and after giving stress condition at 5^{o}C and 35^{o}C.

b. Creaming volume measurement: creams were put into glass beaker and kept at 5^{o} and 35^{o}C each cycle for 12 hours (1 cycle) for 10 cycles then observed changes on each cycle.

c. Viscosity measurement: creams were put into glass beaker then measured viscosity of creams utilizing Brookfield viscometer after that stored at 5o and 35oC each cycle for 12 hours for 10 cycles. After storage at set temperature then re-measured the viscosity of creams and observed cream changes.

d. Inversion phase: creams were put into glass beaker and kept at 5^{o} and 35^{o}C each cycle for 12 hours for 10 cycles then observed emulsion types utilizing dilution method and color dispersion method after that observed cream changes.

e. pH measurement: creams were put into glass beaker and measured pH of creams with using pH meter then kept at 5^{o} and 35^{o}C each cycle for 12 hours for 10 cycles. After that, creams were re-measured pH and observed cream changes.

All data were analyzed statistically with a completely randomized design method.

Antibacterial activity assay

Physical properties of creams such as organoleptic, creaming, viscosity, inversion phase, and pH test were evaluated. Creams, which were stable physically, were subjected to preliminary screening for antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*. The agar diffusion method, using filter paper discs, was used to determine the inhibition zones of snakehead fish cream 2% in mm (3 replications) which consisted of 3 parts i.e. snakehead fish cream 2%, positive control (Amoxicillin), and negative control (cream basis). The petri dishes were incubated under suitable conditions at 37oC for overnight and then the diameters of inhibition growth zones were measured[9].

Results and Discussions

Creams are often made with cream basis oil in water (o/w) type because it is pleasing in appearance, comfortable after use, not oily, spread easily, and easy to clean. Emulsifiers which used in this research are polioksietilen sorbitan monostearate (Tween 60) and sorbitan monostearate (Span 60) with concentrations of 2%, 3%, 4%.

Emulsion type determinations

The results of emulsion type determination utilizing dilution, color dispersion and electrical conductivity method did not show emulsion type changes o/w (Table1.).

Table 1. Emulsion type test

	Emulsion type					
	Before stress condition			After stress condition		
Cream	dilution method	color dispersion method	electrical conductivity method	dilution method	color dispersion method	electrical conductivity
I	o/w	o/w	o/w	o/w	o/w	o/w
II	o/w	o/w	o/w	o/w	o/w	o/w
III	o/w	o/w	o/w	o/w	o/w	o/w

From dilution method, three formulas of cream can be diluted with water (Fig.1).

Fig. 1 Emulsion type test with dilution method

Fig. 1 Emulsion type test with dilution method

This describes three formulas of cream contain the amount of water more on external phase which is based on the external phase in emulsion. Meanwhile, color dispersion method showed that all formula cream gave blue color from blue methylene (Fig.2). It is caused by the amount of water more in formulations so that blue methylene is easy to dissolve in water phase and is able to color all creams.

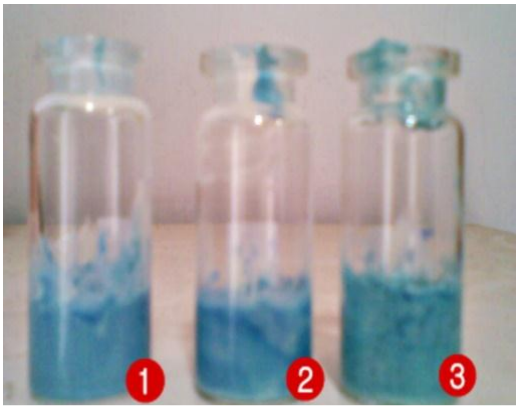

Fig. 2Emulsion type test with color dispersion method

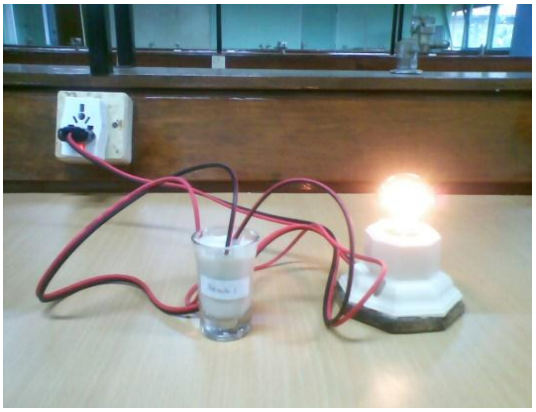

Fig. 3Emulsion type test with electrical conductivity method

On the other hand, electrical conductivity method showed bulb can light (Fig.3) because the amount of water more as external phase which is able to conduct electricity. Based on the results of cream type test can be proved that all creams had emulsion type o/w and showed no inversion phase after stress condition storage.

Organoleptic observations

The odor and color of three formulas of snakehead fish cream before and after stress condition showed no changes of odor and color.

Creaming volume measurement

The observation of creaming volume depict three formulas did not show creaming before and after stress condition (Fig. 4). This can be caused by consistency of cream basis which was thick so that cream was difficult to separate.

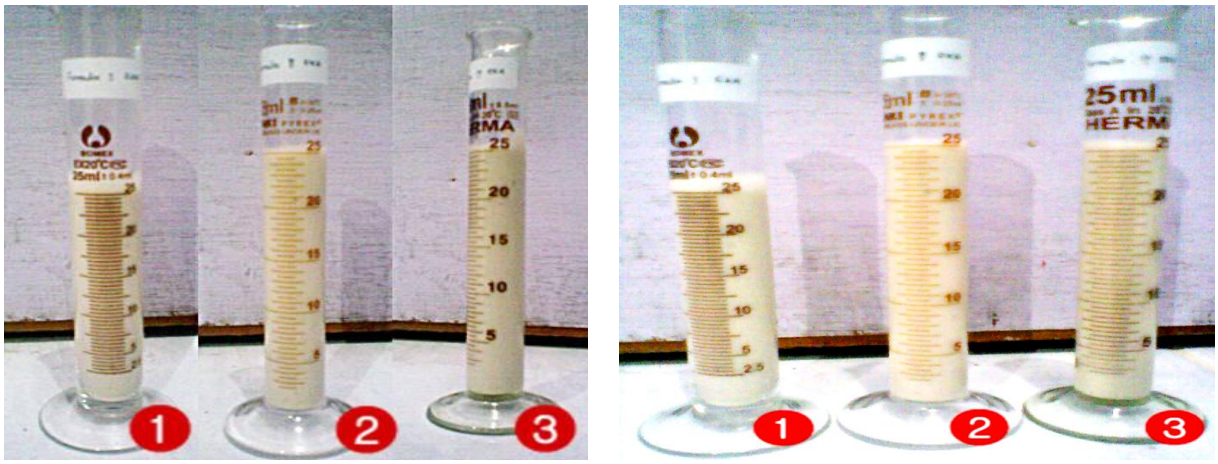

Fig. 4b creaming measurement after stress condition
Note:
1: snakehead fish cream of 2% (tween 60 dan span 60 2%)
2: snakehead fish cream of 2% (tween 60 dan span 60 3%)
3: snakehead fish cream of 2% (tween 60 dan span 60 4%)

Viscosity measurement

The viscosity of creams was measured by Brookfield viscometer using spindle no.5 (Table 2.).

Table2. Cream viscosity

	Viscosity (poise)			
Cream	before stress condition	after stress condition	total	the average
I	52	100		
	52	88		
	56	96		
Total	160	284	444	74
II	96	108		
	88	96		
	104	116		
Total	288	320	608	101.33
III	120	132		
	112	128		
	124	128		
Total	356	388	744	124
Total	804	992		
The average	89.33	110.22		

The results of statistical analysis against viscosity changes of creams showed that there are different concentrations giving a significant influence. This can be seen that F count > F table. It describes the viscosity of creams can be affected by emulsifier concentrations which will increase appropriate the amount of emulsifiers. It can be seen that the viscosity data of creams gave 4% of emulsifier concentration (Tween 60 and Span 60) which was the highest viscosity. Besides that, the viscosity of cream can be affected by temperature which can cause the decrease of cream viscosity. The most emulsion into aqueous, it occurs the temperature increase whereas the low temperature causes viscous emulsion. The cream viscosity also will rise with increasing storage time. This can be seen from the viscosity data after stress condition, creams experienced vigorous viscosity.

pH measurement

The results of pH measurement for three formulas before and after stress condition showed no changes of creams pH having pH around 5.0 – 6.0 based on the skin pH (Table 3.) This explains that no interaction among emulsifier, cream basisand snakehead fish dry extract. Because non-ionic emulsifier i.e. tween 60 and span 60 have neutral charge and low toxicity and irritation. Besides that, this emulsifiers have low sensitivity against pH changes or adding electrolyte and high homogenous degree with other materials[5,8].

Table 3. pH measurement

	pH	
Cream	before stress condition	after stress condition
I	5.54	5.60
II	5.64	5.41
III	5.73	5.52

From all observation results, the variation of emulsifier concentrations of Tween 60 and span 60 did not give significance influence towards organoleptic, cream type and creaming volume but gave influence cream viscosity so that all creams can be stated they were physically stable. After that, all creams, which were stable, were continued by testing antimicrobial activity.

Antibacterial activity assay

Meanwhile, the results of antibacterial activity of snakehead fish cream stated that no inhibition for *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Otherwise, *Bacillus subtilis* had stronginhibition zone with the average diameter 21.6 mm on 3 replications (23, 20, 22 mm).

On the other hand, positive control (amoxicillin 0.1%) had weak inhibition zone for all three bacteria with the average diameter only 6.6 mm on 3 replications (5, 7, 8 mm) and negative control (cream basis) did not give inhibition zone for all three bacteria (Fig. 5).

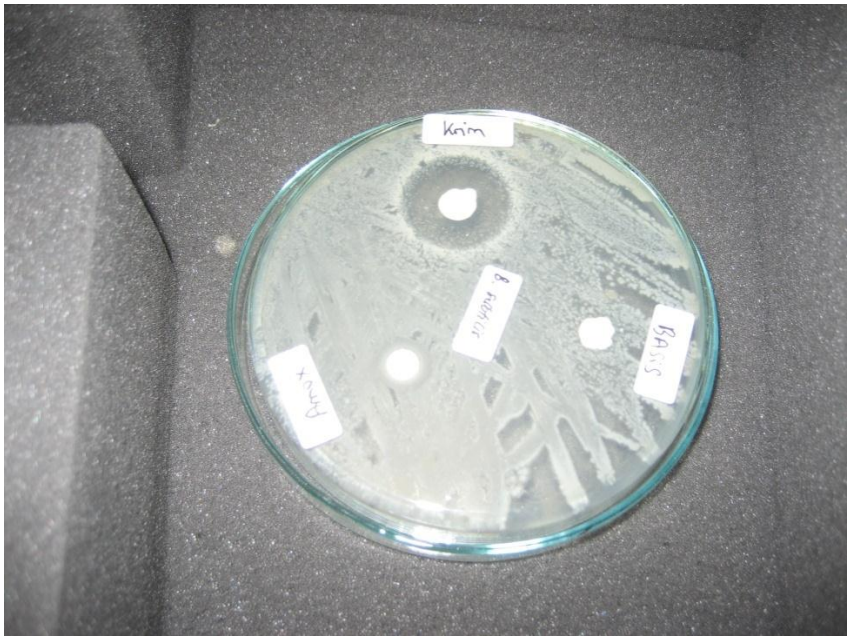

Fig. 5 The inhibition zone of snakehead fish cream 2%

All cream formulations of 2% snakehead fish dry extract with different concentrations can be categorized as physically stable and had antibacterial activity against *Bacillus subtilis*.

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