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Formulation of Microemulsion of Cayenne Pepper (*Capsicum Frutescens* L.) Ethanol Extract and Hair Growth Activity Test

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ABSTRACT

Cayenne pepper contains capsaicin, which exhibits properties that promote hair growth. Microemulsion is an oil and water dispersion system with a particle size of 10-200 nm which can be used topically as a drug delivery system through the scalp to stimulate hair growth. The microemulsion was made with olive oil and tween 80 as surfactants and stirred using a magnetic stirrer at 1000 rpm for 3 minutes. The ethanol extract of cayenne pepper was prepared in microemulsions at three different concentrations: 0.1%, 0.2%, and 0.3%. Microemulsion tests included organoleptic tests, specific gravity, pH, viscosity, freezing and thawing stability tests, and hair growth activity tests on rabbits. The results showed that the microemulsion of cayenne pepper ethanol extract at concentrations of 0.1%, 0.2%, and 0.3% was quite stable with a clear appearance and a particle size ranging from 27.7 nm to 167.8 nm. The microemulsion of cayenne pepper ethanol extract at a concentration of 0.2% was the most effective in enhancing hair growth, achieving a hair growth length of 1.27 over a 30-day period. A two-way ANOVA statistical test showed that the hair length of rabbits using microemulsion was significantly different from the hair length of negative control animals at $p<0.05$.

Keywords: Capsicum; hair growth; microemulsion

INTRODUCTION

Hair loss is a natural phase that happens to everyone because hair has a growth cycle. In general, there is a loss of 50-100 strands of hair a day, but the hair will grow back. If hair loss is more than 100 strands per day and occurs continuously, it is a sign of unhealthy hair (Ide, 2011). Alopecia is a general term for hair loss. Alopecia can occur with or without the formation of scar tissue and can occur at any age (Mentzel & Bierhoff, 2020). Various factors cause alopecia, such as genetic and immunological factors, emotional stress, nutritional deficiencies, hormonal imbalances, post-chemotherapy or radiotherapy, infectious agents, abnormalities of melanocytes or keratinocytes, and neurological factors. Some studies suggest that mutations in keratin may also be linked to the development of alopecia (Shapiro, 2002)

The capsaicin content in cayenne pepper has activity that can stimulate hair growth by activating vanilloid receptor-1 (VR-1), thereby increasing the release of calcium gene-related peptide (CGRP) from sensory neurons. CGRP can increase the production of insulin growth factor-1 (IGF-1) which has an important role in hair growth (Harada et al., 2007). Several studies on the potential of capsaicin for hair growth have shown that capsaicin cream with a concentration of 0.075% can increase vellus hair growth after 21 days in alopecia areata patients (Duque-Estrada et al., 2013). The hair growth of 50 alopecia patients who used 0.065% capsaicin ointment showed an increase compared to the control group after four weeks (Ktiouet, et al, 2009). Extract of capsicum fruit is used in several cosmetic shampoo products at a concentration of 0.1% - 0.3% (Jhonson, 2007).

Microemulsions are thermodynamically stable systems. Dispersion of oil and water is stabilized by the interface layer by surfactant molecules. Microemulsion particle sizes range from 10 to 300 nm, causing microemulsions to appear as clear or translucent solutions. Microemulsions penetrate the skin layers more easily because they have water and oil phases which will affect the permeability of the drug into the skin. Because it has a smaller globule size, microemulsions can improve transdermal drug delivery through the skin (Santos et al., 2008).

This research focused on the formulation and activity test of hair growth microemulsions from the ethanol extract of cayenne pepper (*Capsicum frutescens* L.). This study aimed to prepare a microemulsion formulation as a topical preparation that was both physically and chemically stable and had good hair growth activity.

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Table I. Microemulsion formula of ethanol extract of cayenne pepper

Ingredients	Amount (%)		
	F1	F2	F3
Extract of cayenne pepper	0.1	0.2	0.3
Olive oil	3	3	3
Tween 80	38.5	38.5	38.5
Propylene glycol	10	10	10
Ethanol 96%	3	3	3
Nipagin	0.1	0.1	0.1
Distilled water ad	100	100	100

MATERIALS AND METHODS

Preparation of Cayenne Pepper Ethanol Extract

One kg of cayenne pepper was dried in an oven at 70°C for 3 hours. The dried cayenne pepper was blended with an electric blender. Fifty grams of the dry powder was weighed and extracted with Soxhlet using 500 ml of 95% ethanol for 7 hours. The filtrate obtained was concentrated with a rotary evaporator to obtain cayenne pepper extract. The extract was stored in bottles at room temperature.

Microemulsion Preparation

Microemulsion was formulated using 0.1%, 0.2%, and 0.3% ethanol extract of cayenne pepper as the active ingredient, tween 80 and propylene glycol as surfactants co-surfactant, and olive oil as the oil phase. Distilled water was heated at 75°C, then tween 80 was added into distilled water and stirred using a magnetic stirrer (1000 rpm) at 75°C. Following this, olive oil, propylene glycol, and nipagin was added sequentially with constant stirring using a magnetic stirrer until homogeneous, and microemulsion was formed.

Evaluation of the physical properties of the microemulsion

The microemulsion organoleptic test included visual observation of shape, color, and smell for 8 weeks. The specific weight was measured with a pycnometer, and the pH was measured with a pH meter.

Stability test at room temperature and freeze-thaw evaluation

The stability of the microemulsion was determined by storing it at room temperature for 8 weeks. Microemulsion that did not show separation was considered a stable preparation. Freeze-thaw evaluation was conducted by storing the microemulsion at 4°C for 24 hours followed by storage at 45°C for 24 hours (1 cycle). The organoleptic changes that occurred in each cycle were observed. The test was carried out in 6 cycles.

Centrifugation evaluation

The microemulsion was centrifuged at 3750 rpm for 30 minutes. This centrifugation test demonstrates the stability of the microemulsion due to the equivalent gravitational influence for 1 year.

Determination of globule size

The globule size was determined with the DelsaTM Nano C Particle Size Analyzer. The microemulsion sample was shaken to homogenize, and then the sample was put into the cuvette. The cuvette filled with the samples was inserted into the sample holder and observed at 25°C. The average diameter of the globule size was then observed.

Determination of viscosity and flow properties

The viscosity and flow properties were determined using a Stormer viscometer. The KV value of the tool was determined using glycerin as a Newtonian fluid.

Test for hair growth activity of microemulsion of cayenne pepper ethanol

Preparation of test animals

New Zealand rabbits aged 4-5 months weighing 2.5 kg were housed at room temperature for 10 days to acclimate to the environmental conditions. The experiment was approved by the Ethics Committee of the Faculty of Medicine, Universitas Riau (Project No. 52/UR.16.2/KEP-FK/2022). Three rabbits were acclimatized for a week. The rabbit's back was divided into six areas of treatment with a width of 2.5 x 2.5 cm2 and 1 cm distance between the areas (Figure 1). The next step was shaving the rabbit's hair in these six areas, followed by applying 70% ethanol as an antiseptic. The six treatments were positive control (minoxidil), negative control (microemulsion without extract), F1, F2, F3, and normal control (distilled water). One milliliter of microemulsion was applied (dropped) twice a day (morning and afternoon) to each area of treatment. During the

30-day observation, five rabbit hairs were taken from every area on days 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30.

Data Analysis

The hair length was evaluated statistically using the two-way ANOVA followed by the Tukey test with a significance level of 0.05.

RESULT

All three microemulsion formulas showed a yellow color with the typical aroma of olive oil. The microemulsion organoleptic did not change until 8 weeks of storage (Figure 1). Determination with a pycnometer of the specific weight in the first week and the 8th week of F1, F2, and F3 yielded values of 1.044-1.045 g/ml, 1.044-1.046 g/ml, and 1.043-1.046 g/ml, respectively.

A stability test at room temperature (25°-30°C) showed that all formulas were stable and no separation occurred during 8 weeks of storage. The stability test of the freeze-thaw method was observed at 4°C and 45°C. All formulas do not show phase separation.

All microemulsion formulas were centrifuged at 3750 rpm for 30 minutes at room temperature to maintain clarity and prevent phase separation. The results of the globule size measurements using a particle size analyzer obtained globule sizes of 167.8 nm for FI, 27.7 nm for FII, and 93.6 nm for FIII. All formulas had globule sizes within the range of microemulsion globule sizes, which were 10-200 nm. Each formula had a different polydispersity index value.

The viscosity values of the microemulsion at 1 week were 889.62 cP, 858.12 cP, and 867.83 cP for FI, FII, and FIII, respectively. After 8 weeks of storage at room temperature, the values were 922.80 cP, 981.83 cP, and 904.25 cP for FI, FII, and FIII, respectively. The rheogram curve showed no change in the flow properties of FI, FII, and FIII after 8 weeks of storage at room temperature. All formulas were non-Newton fluids with a thixotropic pseudoplastic flow.

The results of the hair growth activity test showed that the length of hair growth on day 30 for FI, FII, and FIII was 0.84, 1.27, and 1.09 cm, respectively. Meanwhile, the area treated with distilled water showed the shortest hair growth (0.50), and the length of hair in the area on the back of the rabbit as the negative control (microemulsion without extract) was 0.57 cm.

DISCUSSION

In this research, the formulation and test of hair growth activity were carried out from the microemulsion of ethanol extract of cayenne pepper (*Capsicum frutescens* L). The content of capsaicin in cayenne pepper is known to have the activity of hair fertilizer. Capsaicin is a member of the vanilloid family that binds to a receptor called the vanilloid receptor (Gunthorpe et al., 2002). Vanilloid receptors are important for hair growth.

Microemulsion was prepared with different concentrations of cayenne pepper ethanol extract (0.1%, 0.2%, and 0.3%). Microemulsion is a thermodynamically stable oil-in-water (w/o) or water-in-oil (w/o) emulsion with a globule size of 10-200 nm. Microemulsions have been developed for transdermal drug delivery because they can increase the solubility of drugs that are poorly soluble in water (Grampurohit et al., 2011). In general, microemulsions are composed of an oil phase, a water phase, surfactants, and cosurfactants (Maleki Dizaj, 2013). In this study, the oil phase used was olive oil. Olive oil is a vegetable oil that contains medium-chain triglycerides. Oils containing medium-chain triglycerides can produce clearer and more stable microemulsions compared to microemulsions using oils containing long-chain triglycerides (long-chain triglycerides) (Khor et al., 2014). The surfactant used was tween 80. Tween 80 is a non-ionic surfactant that is widely known for its use in topical preparations that is non-toxic and non-irritating to the skin (Kreilgaard, 2002). One surfactant does not sufficiently lower the water-oil interfacial tension to form a microemulsion. It is necessary to add a short-chain amphiphilic molecule or cosurfactant to achieve a zero interfacial tension (Lawrence & Rees, 2012). Propylene glycol is used as a cosurfactant to help reduce interfacial tension as well as stabilize the layer that forms between the two phases (Binarjo & Nugroho, 2014). Ethanol 96% in the formula as a solvent extract also functions as a cosurfactant and as a drug penetration enhancer.

The appearance of microemulsion of ethanol extract of cayenne pepper (*Capsicum frutescens* L) is a clear yellow liquid with a characteristic odor of olive oil and this organoleptic did not change after 8 weeks (Figure 2). Specific weight is the ratio of the weight of a substance to the weight of water with the same volume, all of which are weighed in the air and at the same temperature. The results of the microemulsion specific gravity test at week 1 and week 8 of all formulas showed no significant difference. The specific gravity values of FI, FII and FII at week 1 and week 8 were 1.044-1.045 g/ml, 1.044-1.046 g/ml, and 1.043-1.046 g/ml, respectively. These results indicated that the specific gravity did not change during 8 weeks of storage. The pH measurement showed that the pH

Figure 1. Organoleptic microemulsion ethanol extract of cayenne pepper

Table II. Results of physical evaluation of microemulsion of ethanol extract cayenne pepper

Evaluation	F1	F2	F3
Color	yellow	yellow	yellow
Odor	olive oil	olive oil	olive oil
pH	5,2	5,5	5,5
specific weight (g/mL)	1.045	1,046	1,046
Stability			
Room temperature	stable	stable	stable
Freeze-thaw (6 cycles)	stable	stable	stable
Average globule size (nm)	167,8	27,7	93,6
Polydispersity Index	0,634	0,232	0,250

values for FI, FII, and FIII were 5.1-5.2, 5.3-5.5, and 5.3-5.5 respectively. These findings suggest that the pH range of all formulas is 5.1-5.5 and within the pH range of the scalp.

The microemulsion stability test was carried out at room temperature (25°C-30°C), while the stability test by freeze and thaw method was done at 4°C and 45°C. All microemulsion formulas did not show phase separation during 8 weeks storage. These results indicate that the three formulas are thermodynamically stable, characterized by no physical changes or phase separation.

An evaluation of centrifugation was carried out to provide information about the possibility of phase separation. Centrifugation separates substances based on molecular weight, causing lighter substances rise to the top and heavier substances to settle at the bottom of the container due to gravity. All microemulsion formulas were centrifuged at 3750 rpm for 30 minutes at room temperature to maintain clarity and prevent phase separation.

Determination of globule size using a particle size analyzer showed the globule size of FI, FII and FIII in the microemulsion globule size range (10-200 nm). Each formula exhibited a different polydispersity index value. The polydispersity index describes the variation in the uniformity of the globule size of the sample. A small polydispersity index (less than 0.1) indicates that the sample is monodisperse. The results showed that the polydispersity index value was greater than 0.1, which indicates that the level of uniformity of the globule size distribution is still low. A possible explanation for this finding might be the presence of high fatty acids, which generally causes a negative charge on the droplet surface. This also indicates that there is insufficient electrostatic repulsion formed between droplets, resulting in droplet agglomeration (Shah, 2011).

The viscosity of the microemulsion was tested at week 1 and week 8. The results showed that the viscosity increased after 8 weeks, this is because the formula uses olive oil whose viscosity increases in storage (Rowe et al., 2006). The flow properties of the microemulsion can be seen from the rheogram curve. The flow properties of the microemulsion did not change after 8 weeks of storage at room temperature. All formulas were found to be non-Newton fluids with a thixotropic pseudoplastic flow type because the down flow curve was to the left of the up curve (Figures 3, 4, and 5). Thixotropic flow is a desirable property of

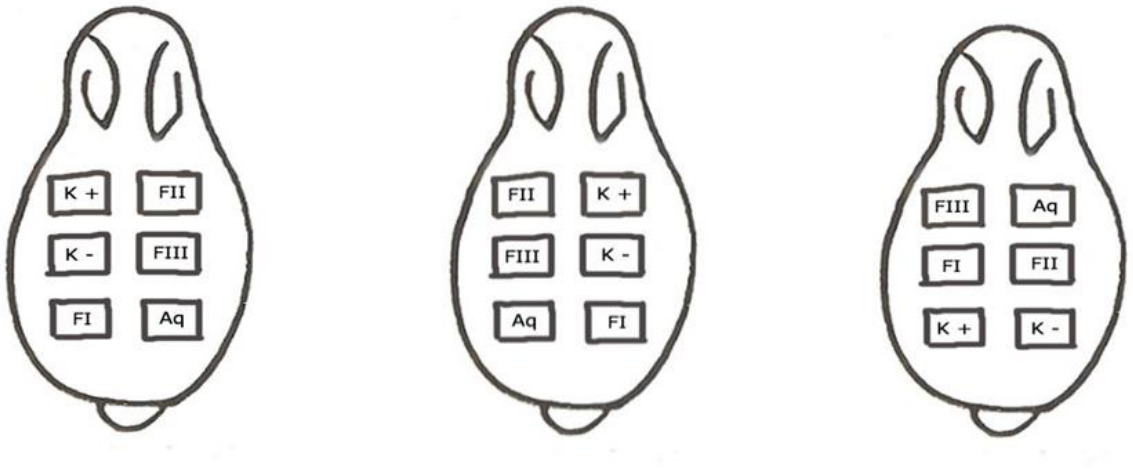

Figure 2. Treatment area on the rabbit's back

Table III. The length of the hair growth of the rabbit

	Hair length (cm) ± SD									
Day	**3**	**6**	**9**	**12**	**15**	**18**	**21**	**24**	**27**	**30**
control +	0±0	0.63± 0.01	0.69± 0.01	0.76± 0.01	0.83± 0.01	0.96± 0.01	1.08± 0.01	1.20± 0.01	1.35± 0.01	1.42± 0.01
control -	0±1	0.19± 0.01	0.22± 0.01	0.25± 0.01	0.31± 0.01	0.36± 0.01	0.42± 0.01	0.48± 0.01	0.53± 0.01	0.57± 0.01
F1	0±2	0.32± 0.00	0.35± 0.00	0.39± 0.00	0.42± 0.00	0.56± 0.00	0.67± 0.00	0.72± 0.00	0.78± 0.00	0.84± 0.00
F2	0±3	0.32± 0.01	0.39± 0.01	0.45± 0.01	0.58± 0.01	0.75± 0.01	0.87± 0.01	1.06± 0.01	1.12± 0.01	1.27± 0.01
F3	0±4	0.32± 0.00	0.37± 0.00	0.42± 0.00	0.58± 0.00	0.69± 0.00	0.75± 0.00	0.89± 0.00	1.03± 0.00	1.09± 0.00
normal	0±5	0.20± 0.03	0.22± 0.00	0.25± 0.00	0.85± 0.00	0.31± 0.00	0.35± 0.01	0.40± 0.00	0.45± 0.00	0.51± 0.00

a pharmaceutical system, where the solution has a high consistency in the container but can be easily poured from the container (Sinko, 2011).

A hair growth activity test was conducted on day 3 to day 30. Observations were conducted by measuring the length of 5 strands of rabbit hair on each part of the rabbit's back. The rabbit's back was divided into six areas of treatment with a width of 2.5 × 2.5 cm^2 and 1 cm distance between the areas. The six treatments were a positive control (minoxidil), a negative control (microemulsion without extract), F1, F2, F3, and a normal control (distilled water). The area on the back of the rabbit treated with distilled water showed the shortest hair growth (0.50), and in the negative control, the area that was given microemulsion without the extract exhibited a hair growth of 0.57 cm. Previous studies have not demonstrated the effectiveness of olive oil as hair fertilizer. However, according to a study conducted by Watanabe et al. (1991), the use of olive oil as hair fertilizer in male rats did not result in hair growth. Hair growth of FI, FII, and FIII on the 30th day of treatment was 0.84, 1.27, and 1.09 cm, respectively.

Microemulsion FII showed more optimal hair growth compared to those of FIII and FI, but its activity was smaller than the positive control (minoxidil), which was 1.43 cm. This might be because FII microemulsion had smaller particle size compared to FI and FII, making it easier for the active substance to penetrate through the skin. The content of capsaicin in cayenne pepper extract can also activate vanilloid receptor-1. This receptor is found in the skin, hair follicles, mast cells, and Langerhans cells. Vanilloid receptor-1 activation by capsaicin causes anagen-catagen transitions in the follicle, and the hair follicle cycle becomes more spontaneous (Bodó et al., 2005).

Based on Table III, all microemulsion formulations in this study were able to promote hair growth better than negative control and normal control. On day 30, the statistical tests showed that the data was distributed normally and

homogeneously, allowing for its further analysis with ANOVA. The results of the two-way ANOVA test between the formula and the length of the treatment showed significant results with a p-value <0.05. Tukey test results showed significant differences in hair length in each treatment with p > 0.05.

CONCLUSION

This research has shown that the ethanol extract of cayenne pepper can be formulated into a microemulsion to form a stable microemulsion during storage. Microemulsion of cayenne pepper ethanol extract 0.2% can enhance hair growth with a length of 1.27 cm after 30 days.

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