

MICROEMULSION FORMULATION OF *ALOE VERA* GEL AND *APIUM GRAVEOLENS* ETHANOL EXTRACT FOR OPTIMIZING HAIR GROWTH PROMOTIONJessie Sofia Pamudji^{1*}, Tri Suciati¹, Lidia², Elin Yulinah Sukandar¹, Irda Fidriani¹¹School of Pharmacy, Institut Teknologi Bandung, Indonesia. ²Department of Pharmacy, STIFI Bakti Pertiwi, Palembang, Indonesia.
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ABSTRACT

Objective: Follicular penetration of hair promotion substances to the bulge region is needed to potentiate cell proliferation and hair shaft lengthening. Nano-size globule is one of important delivery systems applied for hair follicle targeting. This research aims to explore the effect of microemulsion on hair promoting activities of a mixture of hydrophilic substances of *Aloe vera* gel and semipolar substances of *Apium graveolens* ethanolic extract.

Methods: The clear water in oil (W/O) and oil in water (O/W) microemulsions were developed by constructing phase diagram of various ratios of Crodamol GTCC, Croduret 50 SS, glycerin/alcohol, and water. *A. vera* (1%) and celery extract (2%) were incorporated during microemulsion preparation. The microemulsions were applied over shaved skin areas of the backs of Wistar rats, and the average length of 10 hair samples was determined at day 7, 14, and 21 compared to the un-treated area. The weight of hair/cm² area was also analyzed at day 21. Hair growth promotion of the microemulsions was compared to the other groups of rats applied by *A. vera* in water (AVW), celery extract in water and oil (CEW and CEO), and combination of *A. vera* and celery extract in water (AVCEW).

Result: The W/O and O/W microemulsion consisted of globules at the average size of 61.7 and 81.5 nm, respectively, which showed stable size following six cycles of freeze-thaw. The W/O microemulsion increased hair growth dramatically started at day 7 (220%) and day 14 (275%) and slowed-down at day 21 (163%) with similar profile shown by AVW (74.5, 65.8, 31.7%, respectively). On the contrary, the hair growth promotion of O/W microemulsion delayed to day 14 but maintained till day 21 (143.7, 214.5, 171.9 at day 7, 14, and 21, respectively) with similar profile shown by CEW and CEO. Hair densities were also doubled in the areas treated by both microemulsion types than in the areas treated by a combination of AVCEW in water.

Conclusion: Incorporation of hydrophilic and semipolar substances of hair growth promoter in nano-size globules of W/O and O/W microemulsions lead to pilosebaceous targeting and optimize the growth promotion activities.

Keywords: *Aloe vera*, *Apium graveolens*, Water in oil microemulsion, Oil in water microemulsion, Hair growth, Pilosebaceous.

INTRODUCTION

Various medicinal plants have been used traditionally to promote hair growth and prevent hair fall such as *Aloe vera* L. and *Apium graveolens* L. (celery). *A. vera* contains polysaccharide, amino acid, steroid, anthraquinone, various vitamins and minerals [1,2]. *A. vera* usually used as hair promoter, burn wound healing, anti-inflammatory, diuretic, emolien, antipyretic, antibacterial, and laxative [3]. Malic acid and other ingredients of *A. vera* can promote cell proliferation in the hair follicle and improve the hair growth. Celery herb contains flavonoid (apigenin and apiin), phenol, saponin, coumarin, and steroid. Celery herb is traditionally used as diuretic, anti-rheumatic, anti-hypertension, anti-diabetic, and hair promoter [4]. According to Huh *et al.* [5], apigenin, a flavonoid from celery, has an important role in hair elongation.

Follicular penetration of hair promotion substances to the bulge region is needed to potentiate cell proliferation and hair shaft lengthening. Nano-size globule is one of important delivery systems applied for hair follicle targeting. However, large particle size globules cannot penetrate hair follicle so that the efficacy of those substances will not be optimum [6,7] In this research, microemulsion commonly having a globule size in the range of 60-200 nm [8] was proposed for such delivery systems. This research aimed to explore the effect of microemulsion on hair promoting activities of a mixture of hydrophilic substances of *A. vera* gel and semipolar substances of celery ethanolic extract. To optimize hair promotion activity of a combination of these herbals, the formulations were made into different types of oil in water (O/W) and water in oil (W/O) microemulsions.

METHODS**Materials and instrument**

Crodamol GTCC (Croda), Croduret 50 SS (Croda), apigenin, malic acid (Sigma-Aldrich), glycerin, propylene glycol, alcohol, methanol, acetonitrile (Merck), and vitamin C was procured from PT Kimia Farma, aqua biddest.

Analytical balance (Sartorius), water bath, viscometer Brookfield, Delsa Nano-c particle size analyzer Beckmann, magnetic stirrer, oven, refrigerator, centrifuge (Hettich), high performance liquid chromatography (HPLC), Hewlett Packard series 1100, and various common laboratory glasswares.

Tested animal

Healthy white Wistar male rat weighed in the range of 190-220 g was purchased from PT Biofarma.

Simplisia

The celery was collected from Lembang area, and *A. vera* was collected from Cisitua area (Bandung, Indonesia). Determination of both plants was conducted at Bandung Herbarium, Department of Biology, Institut Teknologi Bandung.

First, the roots of celery herbs were removed; then the herbs were washed and cut into small pieces and dried in the oven at a temperature of 60-70°C. Finally, the dried herb was pulverized to get a powder form of celery simplicia.

A. vera leaves were harvested before 8 O'clock in the morning, then peeled and washed to remove the yellow sap. The *A. vera* gel was taken, blended, and filtered. Then the filtrate was heated at a temperature of 56°C for 3 minutes, and 0.5% w/w of ascorbic acid was added to the *A. vera* juice. The juice was finally freeze dried and kept at the fridge until used.

Preparation of celery ethanolic extract

The amount of 470 g of celery powder was refluxed with 2 L of ethanol for 3 hrs and filtered. This process was repeated 3 times and the filtrates were collected and concentrated using rotary evaporator.

Phytochemical screening

Both simplicias were screened for alkaloid, flavonoid, saponin, tannin, quinone, steroid/triterpenoid substances.

Characterization of celery extract and *A. vera* powder

Both preparations were characterized for moisture content, total ash and total water soluble ash, water, and ethanol soluble extract. The content of malic acid in *A. vera* and apigenin in celery extract were also determined.

Assay of malic acid and apigenin

The content of malic acid and apigenin in both O/W and W/O emulsion types, *A. vera* powder, and ethanolic extract of celery were performed employing High Performance of Liquid chromatography. The amount of samples used in the assay was 500 mg of active ingredient, 500 mg of both types of microemulsion, and 10 mg for ethanol extract of celery. All samples were dissolved in 5 ml of methanol. Meanwhile, 10 mg of *A. vera* powder was dissolved in 10 mL of water. Furthermore, 50 µl of each solution was injected to HPLC column. For assay of malic acid content, Kromasil C-18 column, was employed with UV detector at 210 nm. The mobile phase was a mixture of phosphate buffer (pH 2.8) and acetonitrile (99:1) and the flow rate of 1.25 mL/minutes at 25°C. The retention time of malic acid was 6.9 minutes. HPLC condition for assay of apigenin content was as follow: Kromasil C-18 column, UV detector at wavelength of 350 nm, mobile phase was a mixture of methanol and phosphoric acid (58:42), flow rate 1 mL/minute at 25°C. The retention time of Apigenin was 13.7 minutes [9,10].

Formulation of microemulsion dosage form containing celery ethanolic extract and *A. vera*

Preparation of microemulsion

The O/W microemulsion were prepared using Crodamol as an oil phase and a mixture of water, glycerin, ethanol, and Croduret 50 SS as a water phase component. Meanwhile, the W/O microemulsion was prepared using Crodamol and Croduret 50 SS as an oil phase and a mixture of water, glycerin, ethanol, and propylene glycol as the water phase. Each phase was heated in water bath at 50°C and mixed together at 500 RPM for 30 minutes.

During preparation of the micro emulsion, a mixture of glycerin and ethanol were utilized as co-surfactant, therefore the variation of concentration of both co-surfactant were optimized. Optimization of glycerin and ethanol ratios were done in the range of 7:3-3:7, while the optimum ratio of surfactant (Croduret 50 SS) and co-surfactant were also varied in the range of 8:2-2:8. The microemulsions were prepared as mention above and its physical stability were observed for 24 hrs.

Construction of pseudoternary phase diagram

Surfactant was mixed with co-surfactant at the fixed ratio used in the previous optimization process. For W/O microemulsion, water was gradually added to the mixture, which was 1 mL of water for each addition and stirred at 500 RPM for 30 minutes. Meanwhile, for the preparation of the O/W microemulsion, Crodamol GTCC was titrated with 1 mL of water step by step. The observation was started from the formation of clear microemulsion until it formed a cloudy (or separate) microemulsion. All the data obtained from these studies were plotted into a pseudoternary phase diagram.

Incorporation of celery ethanolic extract and *A. vera* into microemulsion base

For the preparation of O/W microemulsion, the celery extract was added into an oil phase, and *A. vera* was added into a water phase. Then each phase was heated at 50°C, mixed together, and stirred at 500 RPM for 30 minutes. For the preparation of O/W microemulsion, the oil phase containing celery extract and the water phase containing *A. vera* powder were heated at 50°C, mixed and stirred for 10 minutes. Then ethanol was added into the mixture and stirred for 30 minutes.

Evaluation of microemulsion

The organoleptic of microemulsion was observed including color, odor, and cloudiness for 4 weeks at room temperature. The physical stability of microemulsion was tested using centrifugation and freeze-and-thaw methods. The centrifugation test was conducted at the rate of 3750 rpm for 5 hrs, and the possibility of phase separation of microemulsion was observed at 1 hr interval.

The stability of microemulsion during 6 cycles of freeze-and-thaw was also investigated for 3 batches of each formula. One cycle consisted of 2 × 24 hrs storage at 4°C and 2 × 24 hrs storage at 40°C. The possibility of phase separation and the alteration of globule size were observed after finishing each cycle.

The globule size of each type of microemulsion was determined utilizing Delsa Nano-C particle analyzer (Beckmann).

Hair growth promotion testing of microemulsion in rat

The study was performed using 6-9 weeks old male white Wistar rats. The hair at the back of rats was shafted at the area of 2 cm × 2.5 cm. Each sample was applied onto the shafted areas to 6 groups with 3 rats/group. The hair growth in the tested areas was observed for 21 days.

The type of preparation used in each group was classified as followed:

Group 1: Solution of *A. vera* powder 1%.

Group 2: Solution of celery extracts in water (CEW) 2%.

Group 3: Solution of celery extracts 2% in oil (CEO) (Crodamol).

Group 4: Solution of a mixture of celery extracts 2% and *A. vera* 1% in water.

Group 5: Microemulsion (W/O) that consists of *A. vera* and celery extract.

Group 6: Microemulsion (O/W) that consists of *A. vera* and celery extract.

Observation of hair growth

The effect of both types of microemulsion to promote hair growth were determined through observation of the existence of hair growth in the area, the length of hair at day 7, 14, and 21 and hair density was measured 21 days after administration. About 10 hairs which grow in each tested area were taken, and the length of hair was measured by a ruler. The hair density was observed visually and also by weighing of hair from each tested area after all hair was cut. All data obtained from this study were analyzed statistically using SPSS with one-way ANOVA and least significant difference.

RESULT AND DISCUSSION

Phytochemical screening was done to identify the presence of the phytochemical compounds in the ethanol extract of celery and *A. vera*

Table 1: Phytochemical screening of *Aloe vera* and celery

Chemical compound	<i>Aloe vera</i>	Celery
Flavonoid	-	+
Saponin	+	+
Tanin	-	-
Quinone	+	-
Steroid	+	+

powder and the result were showed in Table 1, while the characteristic of those preparation were determined through various parameter as showed in Table 2.

In these study, two kinds of microemulsion dosage form were made, namely O/W and W/O microemulsion. Optimization has to be done to choose the best ratio between surfactant and co-surfactant. In the preparation of O/W microemulsion, the mixture of glycerin and ethanol were utilized as co-surfactant. The optimization process was done by preparing microemulsions using a ratio of 7:3 to 3:7 of both substances. It was found that clear and stable microemulsion was obtained from the formula that used glycerin and ethanol within ratio 5:5. Meanwhile, the W/O microemulsion only use glycerin as co-surfactant.

The next optimization process was conducted to choose the best ratio between surfactant Croduret SS with glycerin and ethanol as co-surfactant. The study was conducted in the ratio of 8:2-2:8. The result from these study showed that clear and stable of O/W microemulsion were obtained from the ratio 5:5-7:3. Furthermore, the formula with ratio 5:5 is chosen to develop further because of the concentration of the surfactant was low. Whereas the best formula for W/O microemulsion was showed by the ratio of Croduret SS and glycerin of 7:3. Then ethanol extract of celery and *A. vera* powder were incorporated into the best formula of each type of microemulsion. The final formula for W/O and O/W microemulsion can be seen at Table 3.

Based on the best formula of each type of microemulsion the pseudoternary phase diagram was constructed using water titration method. Data from the study showed that the clear and stable microemulsion were obtained for the concentration of water around 12.28-23.07%, Crodamol GTCC 23.08-35.09%, and a mixture of surfactant-co-surfactant in the range of 48.39-60.34%, respectively.

These data were plot into a pseudoternary phase diagram and the result was showed in Fig. 1.

The content of malic acid and apigenin in both formulas were determined using HPLC method. The result obtain from each formula was shown in Table 4.

According to Huh et al. (2009), malic acid was responsible for cell proliferation in the hair follicle and apigenin has an activity to elongation of hair. Hence, the combination of both active ingredients will have a good hair growth promotion activity.

The organoleptic properties of both type of microemulsions containing 1 % of *A. vera* and 2% of ethanolic extract of celery showed that there were no change in color and odor after storage at room temperature for 4 weeks. The homogeneity of the microemulsion was also good. There was also no phase separation in both types of microemulsion after centrifugation at 3750 RPM for 5 hrs.

In the freeze-thaw test, one vial was used as a control and kept at a temperature of 25°C and the other six vials were used for freeze-thaw stability test. They were kept at 4°C for 48 hrs and then moved to 40°C for 48 hrs. This was one cycle, and the process was continued until six cycles. Based on the results (Table 5), there were no phase separations in both types of microemulsion. Although there was a tendency of increasing the globule size, but it was still in the range of microemulsion globule size. There was also no significant change in pH and viscosity.

The next step was an evaluation of the effect of microemulsion containing *A. vera* and celery extract to promote hair growth in white Wistar male rats. After comparing between the groups treated with both types of microemulsion and the groups treated with solution of celery extract, solution of *A. vera* or the mixture of both extracts, it was found that there were significant differences (p<0.05) in hair growth. The group treated with microemulsion had a longer hair length compared to the group treated with solution dosage form. Hair growth data from each group was shown in Table 6.

Table 2: Characteristic of *Aloe vera* powder and celery

Parameter	<i>Aloe vera</i>	Celery
Water content (%)	15.40	9.00
Total ash content (%)	3.25	7.20
Water soluble ash content (%)	0.68	2.40
Acid insoluble ash content (%)	0.55	0.71
Water soluble extract (%)	26.00	6.12
Ethanol soluble extract (%)	4.00	8.46
Apigenin content (% w/w)	-	0.036
Malic acid (% w/w)	1.44	-

Table 3: Final micro emulsion formula

Name of ingredient	The amount of ingredient (%) in the formula	
	O/W	W/O
Croduret 50 SS	27.5	24.5
Glycerin	27.5	5.25
Ethanol	-	5.25
Celery ethanol extract	2.0	2.0
<i>Aloe vera</i> powder	1.0	1.0
Crodamol GTCC	29.0	12.5
Distilled water	13.0	50.0

W/O: Water in oil, O/W: Oil in water

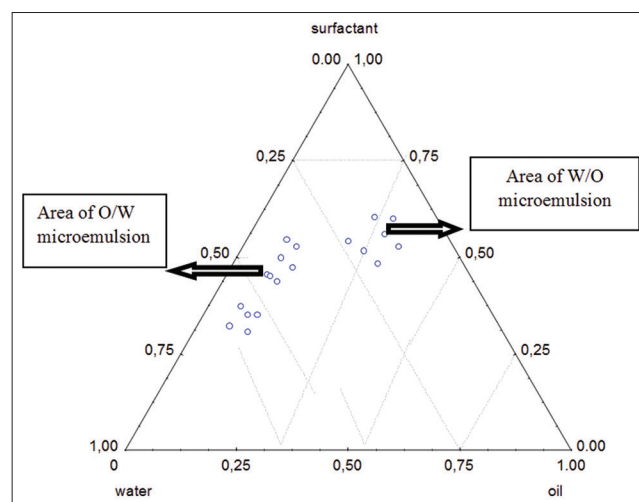


Fig. 1: Pseudoternary phase diagram of surfactant/co-surfactant-Crodamol-water for water in oil and oil in water microemulsion

Table 4: Malic acid and apigenin content in microemulsions

Marker substance content	<i>Aloe vera</i> powder	Ethanol extract of celery	W/O microemulsion	O/W microemulsion
Malic acid (%w/w)	1.44	-	1.37×10^{-2}	1.35×10^{-2}
Apigenin (% w/w)	-	3.55×10^{-2}	6.93×10^{-4}	6.94×10^{-4}

W/O: Water in oil, O/W: Oil in water

Table 5: Physical stability test of microemulsion by freeze and thaw method

W/O microemulsion				O/W microemulsion			
Cycles	Viscosity (cps)	pH	Globule size (nm)	Cycles	Viscosity (cps)	pH	Globule size (nm)
0	9800	5.0	81.5	0	1500	5.5	61.7
1	8500	5.5	84.7	1	1850	6.0	64.0
2	8000	6.0	86.4	2	1200	6.0	68.2
3	7350	5.0	90.4	3	1500	5.0	72.4
4	8500	5.5	92.6	4	1700	5.5	75.6
5	8350	5.0	93.5	5	1350	5.5	77.6
6	8500	5.0	101.1	6	1350	5.0	78.6

W/O: Water in oil, O/W: Oil in water

Table 6: Data of hair growth

Group	Length of hair (mm) at								
	Day 7			Day 14			Day 21		
	Control	Test	T-C	Control	Test	T-C	Control	Test	T-C
AVW	1.83±0.10	3.15±0.24	1.32±0.39	3.80±0.74	6.40±0.19	2.6±0.61	5.23±0.57	7.98±0.57	2.75±0.19
CEW	1.88±0.24	2.95±0.19	1.07±0.27	2.40±0.10	6.30±0.23	3.9±0.32	5.18±0.13	8.25±0.25	3.07±0.23
CEO	1.63±0.10	3.10±0.17	1.47±0.13	2.47±0.51	6.73±1.05	4.26±0.81	4.15±0.27	8.35±0.24	4.20±0.74
AVCEW	2.28±0.10	3.25±0.19	0.97±0.29	3.27±0.21	6.28±0.29	3.01±0.45	4.61±0.20	8.03±0.10	3.42±0.36
W/O	1.33±0.30	4.26±0.12	2.93±0.35	2.50±0.36	9.28±0.23	6.78±0.46	4.53±0.49	11.83±0.71	7.30±0.58
O/W	1.67±0.30	4.07±0.37	2.40±0.36	3.01±0.36	9.37±0.27	6.36±0.42	5.07±0.49	13.68±0.45	8.61±0.56

AVW: *Aloe vera* in water, CEW: Celery extract in water, CEO: Celery extract in oil, AVCEW: Mixture of *Aloe vera* and celery extract in water, W/O: Water in oil microemulsion containing of celery extract and *Aloe vera*, O/W: Oil in water microemulsion containing celery extract and *Aloe vera*

Table 7: Percentage of hair growth in each group compared to control

Group	Percentage of hair growth at day		
	7	14	21
<i>Aloe vera</i> 1% in water	72.13	68.42	52.58
Celery extract 2% in water	57.18	162.50	59.27
Celery extract 2% in oil	128.52	176.25	98.80
Mixture of <i>Aloe vera</i> 1% and celery extract 2% in water	47.30	92.00	74.29
W/O microemulsion	220.00	271.40	161.25
O/W microemulsion	143.70	211.00	169.92

W/O: Water in oil, O/W: Oil in water

Table 8: Hair density in the tested area of each group

Group	Weight of hair per area (g/cm ²)
<i>Aloe vera</i> 1% in water	0.01941
Celery extract 2% in water	0.01320
Celery extract 2% in oil	0.01430
The mixture of <i>Aloe vera</i> 1% and celery extract 2% in water	0.01480
W/O microemulsion	0.03315
O/W microemulsion	0.03310

W/O: Water in oil, O/W: Oil in water

The W/O and O/W microemulsion had globule size at the average of 61.7 and 81.5 nm, respectively. The W/O microemulsion increased hair growth dramatically started at day 7 (220%) and day 14 (275%) and slowed-down at day 21 (163%) with similar profile shown by AVW (74.5, 65.8, 31.7%, respectively). On the contrary, the hair growth promotion of O/W microemulsion delayed to day 14 but maintained till day 21 (143.7, 214.5, 171.9 at day 7, 14, and 21, respectively) with similar profile shown by CEW and CEO. These data may result from the globule size of microemulsion was in nano size, so the active ingredient of *A. vera* can penetrate into stem cells population in bulge area and promote cell proliferation. Besides that, apigenin content of celery extract can promote elongation of hair at anagen phase. Hence,

the activity of active ingredient from *A. vera* and celery extract work simultaneously in promoting hair growth. The last part of these *in vivo* studies was observation of hair density. The weight of hair in tested area of each group was shown in Table 8. Hair density in the tested area that was treated with microemulsions either W/O or O/W type was higher than the other areas.

Based on data from *in vivo* studies, it can be concluded that preparation of *A. vera* and celery extract in the microemulsion dosage forms, either W/O or O/W type, could improve the penetration of active ingredients into pilosebaceous gland and induce a maximum cell proliferation and hair elongation.

CONCLUSION

The O/W and W/O microemulsions containing of *A. vera* 1% and celery extract 2% were prepared utilizing Crodamol GTCC as an oil phase, Croduret SS as surfactant, glycerin and ethanol as co-surfactant and water. The W/O formula consisted of Croduret 50 SS 27.5%, glycerin 27.5%, Crodamol GTCC 29%, while the O/W formula comprised Croduret 50 SS 24.5%, glycerin 5.25%, ethanol 5.25%, Crodamol GTCC 12%. Hair growth in tested areas treated with both microemulsion types was better than the other preparation. The O/W microemulsion showed the highest hair growth promotion activity, indicating that the delivery system of hydrophilic and semi-polar active ingredients in the form of microemulsion was effective to penetrate into the pilosebaceous gland and therefore promoting hair growth.

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