



HAIR GROWTH POTENTIAL COMBINATION OF CELERY (*Apium graveolens* L) AND MANGKOKAN (*Nothopanax scutellarium* Merr) LEAF EXTRACT ON MALE WHITE RABBITS

¹Diah Wardani, ¹Marline Abdassah, ²Yasmiwar Susilawati and ³Anas Subarnas

¹Department of Technology Pharmacy and Cosmetics, Faculty of Pharmacy, Padjadjaran University, Indonesia

²Department of Biological Pharmacy, Faculty of Pharmacy, Padjadjaran University, Indonesia

³ Department of Pharmacology, Faculty of Pharmacy, Padjadjaran University, Indonesia

ARTICLE INFO

Article History:

Received 18th, August, 2016,

Received in revised form 7th,

September 2016, Accepted 12th, October, 2016,

Published online 28th, November, 2016

Key words:

Celery leaves, Mangkokan leaves. Combination, Activity Examination, Thick Extract.

ABSTRACT

Celery and mangkokan leaves are proved scientifically to be able to fasten the rabbits' hair growth, and the activity is linear with hair provision grower or tonic available in the market. This research examined the activity of each leaf extract and combination of the two extracts which was expected to have better activity. The two extracts were obtained by a maceration method of extraction using ethanol 96 %, and the activity test was done with the concentrations of single extract of 2.5%, 5%, and 7.5% and the ratio of each extract of the extract combination of 1:1, 1:2, 2:1, and 2:2. Minoxidil 5% was used as a standard for comparison. The activity test was carried out by rubbing the extracts over the rabbits' back which was cleared from fur twice a day in the morning and in the afternoon for 28 days. The result showed that the combination of the celery and mangkokan extracts with the four ratio above had better activity than the single extract and canine hair tonic in the market. The combination of the celery and mangkokan extracts with the ratio of 1:2 showed the best activity. Irritation and odema observation showed no irritation and odema on the rabbits' back.

Copyright © Diah Wardani *et al.* 2016, This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Hair protects against environment like temperature and ultraviolet. It also protects skin toward bad influence, as temperature controller, sweat evaporation, and sensitive touching sense (Harahap, 2000). Hair is horn planted aslant in the hair follicles. It is known as women's crown. Therefore, having beautiful and healthy hair can make women look beautiful. The beauty and health can be seen from the healthy hair; therefore, head skin and hair need treatment in order to keep them clean and healthy. The problem appears from untreated of head skin and hair such as hair fall (Bariqina & Zahida, 2011). Based on the research that the hair fall is suffered by 50 million people, and 20 millions are women. The causes are various. They are classified by endogen of systemic hormonal diseases, nutrition status, intoxication, and genetic, and exogenous, that is, stimulus from environment or cosmetic use. Hair fall caused by cosmetic and hair dressing can be seen in numbers in African and American women. The use of

rebounder causes hair fall or hair damage is 95% in America and 53% in Africa (Swce et al, 2000; Noruka, 2005). Cosmetic supplies which have activities as the hair grower have been marketed a lot, but in the application sometimes synthesis product like minoxidil can impact local irritation and Eritrea; therefore, herbal products are being more interested (Sawaya,1998).

Today a lot of plants are used empirically to fertilize and grow hair, and the previous research result showed that celery (*Apium graveolens* L) and mangkokan (*Nothopanax scutellarium* Merr) leaf extracts have activities as hair grower /developer (Handojo, 2011, Kuncari, dkk, 2014).

Classification of celery (*Apium graveolens* L) Kingdo: Plantae; Divisi: Magnoliophyta, Class: Magnoliopsida, Ordo: Apiales, Familia : Apiaceae, Genus: *Apium*, Spesies: *Apium graveolens* L. Classification, mangkokan (*Nothopanax scutellarium* Merr.): Kingdom: Plantae, Divisi: Magnoliophyta, Class: Magnoliopsida, Ordo:

*Corresponding author: Diah Wardani

Department of Technology Pharmacy and Cosmetics, Faculty of Pharmacy, Padjadjaran University, Indonesia

Apiales, Famil: Apiaceae, Genus: *Polyscias*, Spesie: *Polyscias scutellaria* (Burm.f.) Fosberg (Cronquist, 1981; Backer, 1963).

Classification Based on the cause, classification of hair loss can be divided into: congenital, abnormal hair growth cycle, the hair shaft abnormalities, medications, hormonal disorders, trauma, infection, and disease with destructive process. Hair loss or alopecia caused by mechanical trauma can be divided into three types, namely: i) Traumatic alopecia: Alopecia hair loss due to trauma has an area demarcated and is the commonest cause cicatricial alopecia; ii) Alopecia due to pressure : the pressure of time, for example in patients who lie long can cause ischemia, necrosis, and ulceration in the scalp. This situation results in hair loss developing into cicatricial alopecia which are generally irreversible; iii) Traction Alopecia: Chronic traction can cause atrophy of hair follicles with follicular inflammation and broken hair causes hair loss through alopecia locals. This situation can be found in teenage girls with pigtailed ponytail tight, youths and children of Afro-pigtails-pigtails the Caribbean with little hair as well as on the state of trichotillomania (Suling, 2010).

Hair loss is a condition in which the occurrence of hair loss in the range of approximately 100 strands per day (Soepardiman, 2010). Hair loss is a disturbance or disorder where hair is detached from the scalp or skin thereby disrupting a variety of biological functions of the body hair (Sinclair, 2009). In the previous research, had been known that the celery leaf extract was reported to have a optimum growing effect with an emulsion preparation at the concentration of 7.5% (Putra, 2013). The gel preparation of celery extract and apigenin contained in the celery leaves give better hair growth on mice compared to control (Kuncan, dkk, 2014). Furthermore, the celery herbal extract has optimum growing effect (Kusumastuti, 2007) at the concentration of 7.5% and the preparation of microemulsion had growing effect at the concentration of 10% (Tambunan, 2012).

In literature it was reported that flavonoid compound is a vasodilator (Nour & Nur, 2012) that it can fasten the hair growth. The mechanism of vasodilator is equal to minoxidil. Minoxidil is the hair grower preparation available in the market and its mechanism of action is to increase blood flow to papilla dermal (Headington, 1987).

RESEARCH METHODOLOGY

Materials

The materials are fresh celery and mangkokan leaves picked in March 2016. The celery leaves were picked from Mekar Jaya Subdistrict, Cigunung Village, Rancabango, Garut, West Java, but mangkokan leaves were picked from Sukawening, Pamekarsari, and Sukaratu subdistricts, Garut Regency; 96% ethanol, 5% minoxidil (positive comparative control) and New Zealand white male rabbits were taken from Asep Rabbit's Rabbit ranch in Lembang, West Java, Indonesia with certificate no 2398/2016.

Apparatus

Macerators, Evaporators, Water bath, glassware (Pyrex) electronic balance, porcelain saucer, stove, flannel cloth, scissors, razor, pins, cello tip, digital push compasses.

Research story

The research was agreed by the Health Research Ethics Committee of Faculty of Medicine of Padjadjaran University, No 903/UN6.C1.3.2/KEPK/PN/2016.

The plants collected were determined by the Faculty of Biology, Plant Taxonomy Laboratory Padjadjaran University, No HB /04/2016, and also in Herbarium of biological Science and Technology of Bandung Institute of Technology, No 377/II.CO2.2.PL.2016. For the research purposes, the celery and mangkokan plants were examined completely.

Extract production processes

Celery and mangkokan leaves were dried under direct sun ray. After they were really dried, they were powdered, and each was extracted. The leaves powder of 1000 grams was sunk in 3000 ml Ethanol 96% then kept for 3x24 hours, by changing ethanol every 24 hours and stirred. Then it was filtered by using flannel cloth; then the filtrates were kept (Filtrates A). The remain deposit was sunk in 3000 ml ethanol 96% for one day and stirred and filtered by flannel cloth to gain Filtrate B, up to the third day, Filtrate C was gained. Filtrate A and B were mixed with Filtrates C then let it be for a night so that the dissolver evaporates, to thicken the extract, evaporator was used; then kept it in on the waterbath for many hours to make it more perfect. Each extract gained was kept in extract bottles.

Test supply processes

Celery leaf extract was taken 2.5, 5, and 7.5 grams plus ethanol 96% up to volume 100 ml (concentration 2.5, 5, and 7.5). The same way was taken for mangkokan leaf extract. After each was tested, the better activity examination was gained, the two concentration show the better hair growth was concentration 7.5%.

Extract combination production process with some comparison series

1. Combination of celery and mangkokan leaf extracts with ratio 1:1, was made by extracting celery and mangkokan leaves concentration 7.5% taken for 7.5 grams the thick celery extract mixed with 7.5 grams of mangkokan extract then was added ethanol 96 % up to 100 ml.
2. Combination of celery and mangkokan leaf extracts with ratio 1:2, was made by concentration of celery and mangkokan leaves extract concentration 7.5% taken for 7.5 grams of thick celery extract mixed with 15 grams of mangkokan leaf extract and ethanol 96% up to volume 100 ml.
3. Combination of celery and mangkokan leaf extracts with ratio 2:1 was made by extracting celery and mangkokan leaves with concentration 7.5% taken 15 gram thick celery leaf extract mixed with mangkokan leaf extract for 7.5 gram and ethanol 96% up to volume 100 ml.

- Combination of celery and mangkokan leaf extracts with ratio 2:2 was made by extracting celery and mangkokan leaves with concentration 7.5 % taken for 15 grams of thick celery leaf extract and ethanol 96 % up to volume 100 ml.

Activity test Supplies in fastening a rabbit's hair

The activity tests of celery and mangkokan leaves towards the growth of male rabbits' hair by using Tanaka *et al*'s method (1980). The rabbits' back fur was cleared or cut clean, and separated into four parts, each forms rectangular 2x2.5 cm, and the distance between areas was 1 cm. After fur cutting, and before rubbing, the target areas were rubbed by ethanol 96% as antiseptic.

The activity tests were made for three rabbits (triplet) by treating the same test, the purpose was to compare whether the rabbits' hair growth the same or not with the same species and ages.

Test applied

- Without using anything as normal control
- Ethanol 96% as negative controller
- Minoxidil 5% as positive controller
- Celery leaf extract, concentration 2.5%
- Celery leaf extract, concentration 5%
- Celery leaf extract, concentration 7.5%
- Mangkokan leaf extract, concentration 2.5%
- Mangkokan leaf extract, concentration 5%
- Mangkokan leaf extract, concentration 7.5%
- Combination extract 1:1
- Combination extract 1:2
- Combination extract 2:1
- Combination extract 2:2

Before being treated, the rabbits were adapted first for a week in order not to make them distress. The rubbing was made in the second day in the morning and in the afternoon for 1 ml in each part. The first day of rubbing was considered 0 day. The observation was conducted for 28 days. The determination of rubbing areas, one rabbit with for tested areas. The observation was by pulling 10 pieces of rabbits' hair in the 28th day, then they were stuck on cello tip. They were measured by using digital push compasses. The test is performed on 3 rabbits (triplo), in this study there are 13 testing means rabbit that I used in this study was a total of 39 rabbits.

Data analysis

Having obtained the data from the research, the data processing is done by using statistical analysis deskriptif because it deals with how data can be depicted, described or inferred either numerically or graphically. For getting an overview of the data so lbh readable and meaningful (Purwandari, 2012).

RESULTS AND DISCUSSION

Maceration

The materials used in the research were celery and mangkokan leaves. The materials collected were cleaned and dried under direct sun ray for rate 3-5 days. The purpose of drying was to lessen water rate so that the organism fungi and microorganism can be avoided, to stop

enzymization, and to prevent chemical changes; therefore, they can be kept without damages for a long time. Dried samples were powdered in order to extend the target areas so that the contact between samples surfaces and dissolved fluid were larger. The method used in this research was macerations because it was the simplest method; that is, to sink the materials in the essence dissolve. This method has some advantages, such as, the working process and apparatus are simple and fit to compound which cannot keep the heat. However, this method has disadvantages; that is, it needs much time and used much essence. In maseration by stirring so often to prevent thickness so that active substance can be extracted maximally. Rudiment linked with much or little which can be extracted by ethanol 96% in mangkokan leaves is more than those of celery leaves.

Table 1 The result of samples extract of celery leaves (*Apium graveolens* L.) and mangkokan leaves (*Nothopanax scutellarium* Merr.)

Sample names	Sample Weight	Extract Weight	Rendemen
Celery leaves	2100 gram	371,20	17,67
Mangkokan leaves	2000 gram	358,50	17,92

Study examined extracts parameters to determine the quality of the extract of the physical properties and chemical content. Tests done organoleptik include inspection, and water content. Results can be seen in table 2.

Table 2 The results of extract quality parameter

Extract Quality Characteristic	Parameter	Result	
		Celery Leaf Extract	Mangkokan Leaf Extract
Organoleptik	Form	Thickness	Thick
	Color	Dark green	Brownish green
	Smell	Special celery	Special mangkokan
Water rate (%)	Test result	0,5	0,4
	Standar MMI	8	10

Screening phytochemical carried out on botanicals and extracts concentrated celery (*Apium graveolens* L.)

Table 3 Sample screening results of phytochemistry, celery leaf (*Apium graveolens* L.) and mangkokan leaf (*Nothopanax scutellarium* Merr.) extracts

Samples	Compound Group	Examination day	
		Sample powder	Thick extract
Celery Leaves	Alkaloid	-	-
	Steroid/Triterpenoid	+	+
	Saponin	+	+
	Flavonoid	-	-
	Tanin	-	-
	Kuinson	-	-
	Alkaloid	-	-
Mangkokan Leaves	Steroid/Triterpenoid	+	+
	Saponin	+	+
	Flavonoid	-	-
	Tanin	-	-
	Kuinson	-	-
	Alkaloid	-	-

Note: + : Detected, - : Undetected

and leaves mangkokan (*Nothopanax scutellarium* Merr) to determine the secondary metabolites contained in the extract obtained. Results can be seen in table 3.

Activity test referred to Tanaka’s method (Tanaka *et al*, 1980). Before being treated, the rabbits were adapted first for seven days so that the rabbits would not distress which could affect the fur growth. During adaptation the rabbits were treated the same. For comparison in the research, minoxidil 5% available in the market were used. It enlarges vessels with have the same mechanism with flavonoid (Nour *et al*, 2012), and flavonoid content was found in the celery and mangkokan leaves by phytochemistry screening identification. From the measurement data, the hair length collected than measured the growth average shows in table 4.

Table 4 Data extract combination activity in accelerating hair growth

Treatment group	Concentration	Hair Length (mm)	Statistical reports
Normal control	-	9,09	9,09 ±0,389
Alcohol	96%	9,01	9,01 ±0,493
Minoxidil	5%	13,35	13,35 ±0,666
Celery leave extract	2.5%	10,53	10,53 ± 0,54
	5%	11,67	11, 67 ± 0,965
	7.5%	12,13	12,13 ± 0,727
Mangkokan leave extract	2.5%	10,22	10,22 ± 0,373
	5%	11,02	11,02 ± 0,493
	7.5%	11,45	11,45 ± 0,350
Combination extract	1:1	11,25	11,25±0,548
	1:2	15,36	15,36±0,390
	2:1	14,25	14,25±0,387
	2:2	14,33	14,33±0,315

Notes : Normal control = without treatment
 Alcohol 96% = negative control
 Minoxidil = positive control

Concentration : 2.5% = 2.5 mg/lt
 5% = 5 mg/lt
 7.5% = 7.5 mg/lt

Concentration combination :

1:1 = 15 mg/lt
 1:2 = 22.5 mg/lt
 2:1 = 22.5 mg/lt
 2:2 = 30 mg/lt

Definition of the combination :

1:1 = 1celery : 1 mangkokan
 1:2 = 1 celery: 2 mangkokan
 2:1 = 2 celery: 1 mangkokan
 2:2 = 2 celery: 2 mangkokan

In table 4 you can see the different length of hair growth after 28 days, the result of normal and negative controls of long hair length growth which grew in 28 days is the same, it means that alcohol that was used as disolvant in the research had no pharmacological activities which could grow hairs.

Activity test of hair growth towards each extract could produce the best hair growth in the extract with concentration 7.5% both for celery leaf extract or for mangkokan that the concentration of 7.5% can become basic concentration which will be combined.

The celery and mangkokan leaf extracts is proved to have activities to grow hair but it is less than positive control (minoxidil 5%). The combination of celery and mangkokan leaf extracts has better activities than a single extract, but from the four tested combinations, the best activity test was 1:2 combination, even the gained result from the two combination was more than the activity of hair grower of minoxidil 5%. The combination result produced maximum hair growth activities. It was assumed that there was pharmacological effect which synergy each other between the two extracts combined. The two extrracts contained flavonoid that it interacted to produce the wanted effect.

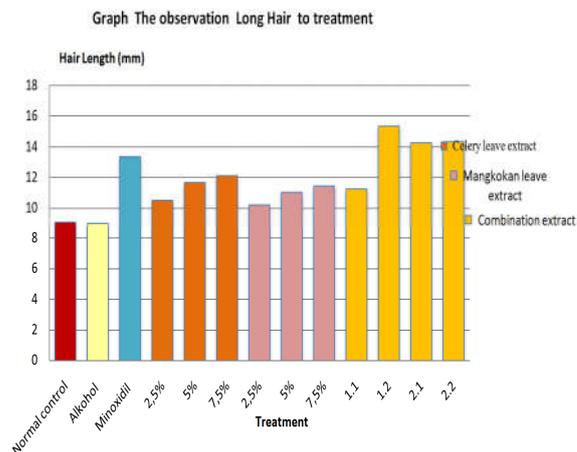


Figure 1 Graph The observation Long Hair to treatment

The above graph shows the result of hair length from every treatment performed. It shows the length of rabbits’ hairs after being observed for 28 days.

Table 5 Irritation and edema (Skin value classification)

Kind			
Eritema	Value	Edema	Value
No eritema		No Edema	
Little eritma		Odema is very light	
Eritema is clear		Odema light (side & clear enlargement	
Eritema moderate to strong		Odema moderate (thickness ±1 mm)	
Eritema worst and wounded		Odema worst (thickness >1 mm)	

Notes: Measurement range from 0 to

Table 6 Observation results of irritation and edema

NO	Treatment	Observation day	Irritation	Edema
1	Mangkokan 2,5%	0	0	0
		7	0	0
		14	0	0
		21	0	0
		28	0	0
		0	0	0
2	Mangkokan 5%	7	0	0
		14	0	0
		21	0	0
		28	0	0
		0	0	0
		7	0	0
3	Mangkokan 7,5%	14	0	0
		21	0	0
		28	0	0
		0	0	0
		7	0	0
		14	0	0
4	Celery 2,5%	21	0	0
		28	0	0
		0	0	0
		7	0	0
		14	0	0
		21	0	0
5	Celery 5%	28	0	0
		0	0	0
		7	0	0
		14	0	0
		21	0	0
		28	0	0
6	Celery 7,5%	0	0	0
		7	0	0
		14	0	0
		21	0	0
		28	0	0
		0	0	0

		0	0	0
		7	0	0
		14	0	0
7	Combination 1:1	21	0	0
		28	0	0
		0	0	0
		7	0	0
		14	0	0
8	Combination 1:2	21	0	0
		28	0	0
		0	0	0
		7	0	0
		14	0	0
9	Combination 2:1	21	0	0
		28	0	0
		0	0	0
		7	0	0
		14	0	0
10	Combination 2:2	21	0	0
		28	0	0
		0	0	0
		7	0	0
		14	0	0
12	Alcohol 96%	21	0	0
		28	0	0
		0	0	0
		7	0	0
		14	0	0
13	Minoxidil 5%	21	0	0
		28	0	0

This observations no rabbit who experience irritation or edema, so that all the extracts tested are safe and can be applied in human scalp as a hair grower.

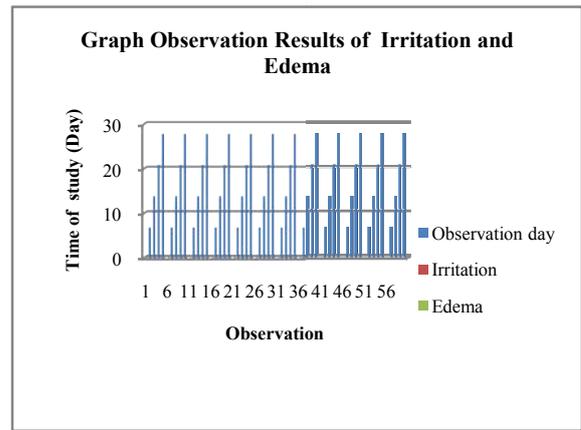


Figure 2 Graph observation results of irritation and edema

Because there are no irritation and edema, the graph shows the permanent and stable results, irritation and edema are not found in all tests performed.

Table 7 The observation of rabbit hair celery leave extract

Observation day	Concentration		
	2,5%	5%	7,5%
0			
7			
14			
21			
28			

Figure on **tabel 7** s the result of observations made during the 28 days, an activity test on concentrated extract of celery with variations in concentration of 2.5, 5, and 7.5.

Figure on **tabel 8** the result of observations made during the 28 days, an activity test on concentrated extract of mangkokan with variations in concentration of 2.5, 5, and 7.5.

Tabel 8 The observation of rabbit hair mangkokan leave extract

Observation day	Concentration		
	2,5%	5%	7,5%
0			
7			
14			
21			
28			

Tabel 9. The observation of rabbit hair treatment control

Observation day -	Concentration		
	Without Treatment	Alkohol 96%	Minoxidil 5%
0			
7			

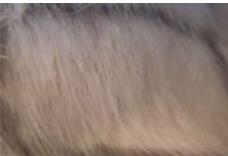
Tabel 9. The observation of rabbit hair treatment control

Observation day -	Concentration		
	Without Treatment	Alkohol 96%	Minoxidil 5%
14			
21			
28			

Figure on **tabel** the result of observations made during the 28 days, an activity test on negatif control and positif control.

Figure on tabel 10. the result of observations made during the 28 days, an activity test on concentrated combination extract.

Tabel 10 The observation of rabbit hair treatment combination extract

Observation day	Concentration			
	Combination 1	Combination 2	Combination 3	Combination 4
0				
7				
14				
21				
28				

CONCLUSION

The activity test of male rabbits' fur/hair using celery leaf and mangkokan leaf extract is better than those of their single extracts. The combination of celery and mangkokan extracts which affects better than that of minoxidil 5% available in the market. Combination of celery and mangkokan leaf extract 1:2 shows the best effect of other combinations. Irritation observation is very excellent because there is no irritation nor edemas in the activity test of single or combination extracts. flavonoids contained in the combination of extracts work in synergy to produce a pharmacological activity maximum.

References

1. Backer, CA., Barhuizen van den Brink, Jr.R.C. 1963. Flora of java. Vol 1, N.V.P. Noordhoff, Groningen, the Netherlands. P.387
2. Bariqina, E., Zahida, I. 2011. Hair Care & Styling. Yogyakarta: Adicita Karya Nusa Indonesia.
3. Cronquist., Arthur. 1981. An integrated System of Classification of Flowering Plants. Columbia University Press: New York. P 838-839.
4. Handojo, Y.2011. Stability Test and Physical Activity White Rat Hair Growth Gel Extracts of preparations mangkokan (*Nothopanax Scutellarium Merr*). Jakarta: University Indonesia Press.P.1-28.
5. Harahap, M. 2000. Dermatology, Volume I. Jakarta. Indonesia : Hipokrates
6. Kuncari, SE., Iskandarsyah., Pratiwi. 2014. Irritation Test and Hair Growth Activity Rats: Effects Gel preparations Apigenin and Herba Celery (*Apium graveolens Lin*). Jakarta: University Indonesia Press.P.15-20.
7. Headington, J.T. 1987. Hair Follicle Biology and Topical Minoxidil: Possible mechanism of Action: Dermatologica Vol 175: Karger Medical and Scientific Publisher. P.1-4.
8. Kusumastuti .A. 2007. The Effect of Water Juice of celery (*Apium graveolens L.*) on the Growth Rabbit Long Hair Males. Yogyakarta: University Ahmad Dahlan Press. Indonesia.
9. Noruka, NE.2005. Hair loss: is there a relationship with hair care practices in nigeria? *International Journal of Dermatology*.
10. Nour AS, Nur P.2012. Mechanism of Tea Mistletoe Action on Blood Vessels. *Journal Medical Brawijaya*, Ind.onesia Vol. 27.
11. Putra, PT. 2013. Formulation and Testing Effectiveness performed Emulsion Hair Growth Stimulants Extract Celery (*Apium graveolens Lin*). Bogor: University of Pakuan Indonesia Press.P.5-19.
12. Sawaya, ME. 1998. Novel Agents for the Treatment of Alopecia. Seminar in Cutaneous Medicine and Surgery. Miami. W.B Company.
13. Suling, PL. 2010. Hair Fall: Cosmetic Dermatology Update. Simposium National, Exhibition and Training Cosmetic Dermatology.
14. Sinclair, R., Jolley, D., Mallari, R., Magee, J.2004.The reliability of horizontally sectioned scalp biopsies in the diagnosis of chronic diffuse telogen hair loss in women. *J Am Acad Dermatol*; 51:189-99.
15. Soepardiman, L. 2010. Hair abnormalities. Jakarta: Faculty of Medicine University Indonesia Press.
16. Swce, W., Klontz, KC., Lambert, LA. 2000. Nation wide of alopecia associated with the use of a hair-relaxing formulation. *Arch Dermatol*.
17. Tambunan, LR. 2012. Stability Test microemulsion and microemulsion Celery Leaves Urang Aring and Effectiveness against Rat Hair Growth Males Sprague Dawley. Jakarta: University Indonesia Press.P.26-43
18. Tanaka, S., Saito, M., Tabasa, M., 1980, Bioassay of Crude Drugs for Hair Growth Promoting Activity in Mice by a New Simple Method, *Planta Medica*, Japan.P. 84-90
19. <http://eka-purwandari.blogspot.com/2012/06/artikel-statistik-deskriptif.html>
