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## Irritation Studies and Evaluation of Hair Growth Activity of Combination of Ethanol Extracts from *Ocimum basilicum* L. and *Morinda citrifolia* L. Leaves



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### ABSTRACT

The aim of present study was to evaluate the hair growth activity of combination of extracts of *Ocimum basilicum* L. and *Morinda citrifolia* L. The plant extracts were obtained by maceration method using ethanol 70%. Extracts were combined with the composition: a combination of 1 (ethanol extract of leaves of basil concentration of 1% and 4% noni leaf), a combination of 2 (ethanol extract of leaves of basil concentration of 2.5% and 2.5% noni leaf), and combinations of 3 (ethanol extract of the leaves basil concentration of 4% and 1% noni leaf). Hair growth Activities were expressed as the rate of hair growth for 28 days. The results indicated that combination 3 have the longest hair; an average length hair on rabbits with hair growth rate value 20.22 mm.



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## INTRODUCTION

Hair serves as a protective head and a healthy hair can boost person's self-confidence. One characteristic of unhealthy hair is hair loss, which is characterized by losing more than 100 hairs per day continuously. Normally hair will fall as much as 50-100 hairs per day and will grow back and replaced with the new hair.

Hair growth has three phases, namely the anagen (growth period), catagen (atrophy period) and telogen (resting periods). Each phase has a different time period. The duration of the anagen period ranging from 2 to 6 years with a growth rate ranging between 0.03 to 0.045 mm per day, catagen duration of 2 to 3 weeks, and the telogen lasts 2 to 3 months, in humans.

Hair loss can be prevented by performing hair treatment to nourish hair and overcome hair loss. Ingredients of cosmetic products to prevent hair loss are available in the market; a few still derived from synthetic substances such as minoxidil, these materials fall into the category of drugs, not intended for use as cosmetics and can cause termination hypersensitivity and its use will cause the loss back.

Some plants can be used to treat hair loss includes basil and noni leaves. The content of tannins and flavonoids from basil leaves suspected to play a role in accelerating the growth of hair. Based on the research that has been done, the ethanol extract of noni leaf 10%, can be used in the process of wound healing and showed growth of hair on the skin of mice that have been hurt, their active compounds such as saponins, triterpenes, tannins, alkaloids, iridoid glycosides and flavonoids, works as an antibacterial, astringent and antioxidant.

## MATERIAL AND METHOD

### Collection and identification of plant

Plant material of *Ocimum basilicum* L. (leaves) and *Morinda citrifolia* L. (leaves), were collected in the month of December from the region of Bogor, West Java. The plants identified in Herbarium Bogoriense, Research Center for Biology, Indonesia Institute of Sciences, Cibinong, West Java.

### Formulation

Formulations were prepared by fusion method, with a combination:

1. Combination 1: 1 % <sup>w/v</sup> of ethanol extract *Ocimum basilicum* L. (leaves) and 4% <sup>w/v</sup> of ethanol extract *Morinda citrifolia* L. (leaves) in ethanol 70%.
2. Combination 2 : 2,5 % <sup>w/v</sup> of ethanol extract *Ocimum basilicum* L. (leaves) and 2,5% <sup>w/v</sup> of ethanol extract *Morinda citrifolia* L. (leaves) in ethanol 70%.
3. Combination 3: 4% <sup>w/v</sup> of ethanol extract *Ocimum basilicum* L. (leaves) and 1% <sup>w/v</sup> of ethanol extracts *Morinda citrifolia* L. (leaves) in ethanol 70%.

### Primary skin irritation test

The institute has been cleared for the said biological evaluation by Animal Ethics Committee (Letter No.317/IV/HREC/2016). A healthy male white rabbit New Zealand were used, weighing about 2 kg and aged 4-5 months were selected for the study. The hair from the dorsal area of approximately 10 x 15 cm or less 10% of the body surface to place the exposed to the test preparation. Sharing area starting from the scapula (shoulder) to the ilium (hip bone) and a half down the body on each side. 1 ml Test samples (blank, combination 1, 2 and 3) were applied on the skin of rabbits, then covered with a sterile gauze and glued together with plaster then wrapped with a bandage, and let stand for 1 hour. After 1 hour plasters and bandages opened and then observed for erythema and edema for 48 hours after application. Data were analyzed to obtain primary irritation index.

**Table 1 Evaluation of Skin Reaction**

No	Erythema		Edema	
	Type	Score	Type	Score
1	No erythema	0	No edema	0
2	Very slight erythema (barely perceptible)	1	Very slight edema (barely perceptible)	1
3	Well-defined erythema	2	Slight edema	2
4	Moderate to severe erythema	3	Moderate edema	3
5	Severe erythema (beet redness) to slight eschar formation (lesion in depth)	4	Severe edema	4

**Table 2 Category for Primary Irritation Reaction in Rabbit**

No	Category for Reaction	P.I.I.
1	Non-irritant	0 – 0.4
2	Slightly irritant	0.5 – 1.9
3	Moderately irritant	2 – 4.9
4	Strongly irritant	5 - 8

### Hair growth activity test

Model by Tanaka was used for the study of hair growth with slight modification. Three rabbits were used. Before test, the back of rabbits shaved to remove all the hair and then divided into 5 parts, 3 parts on the right and 2 parts left. Then 1 ml each test sample (blank, 2% minoxidil, combination 1, 2 and 3) was applied, twice a day. This treatment was continued for 28 days. During the course the hair growth pattern was observed qualitatively and recorded.

### RESULTS AND DISCUSSION

**Table 3 Scoring Result of Skin Reaction**

No	Sample	Erythema Score			Edema Score		
		Observation time (hour)			Observation time (hour)		
		24	48	72	24	48	72
1	Blank	0	0	0	0	0	0
2	Minoxidil	0	0	0	0	0	0
3	Combination 1	0	0	0	0	0	0
4	Combination 2	0	0	0	0	0	0
5	Combination 3	0	0	0	0	0	0
	Total	0	0	0	0	0	0
	<b>PII</b>	<b>0</b>					

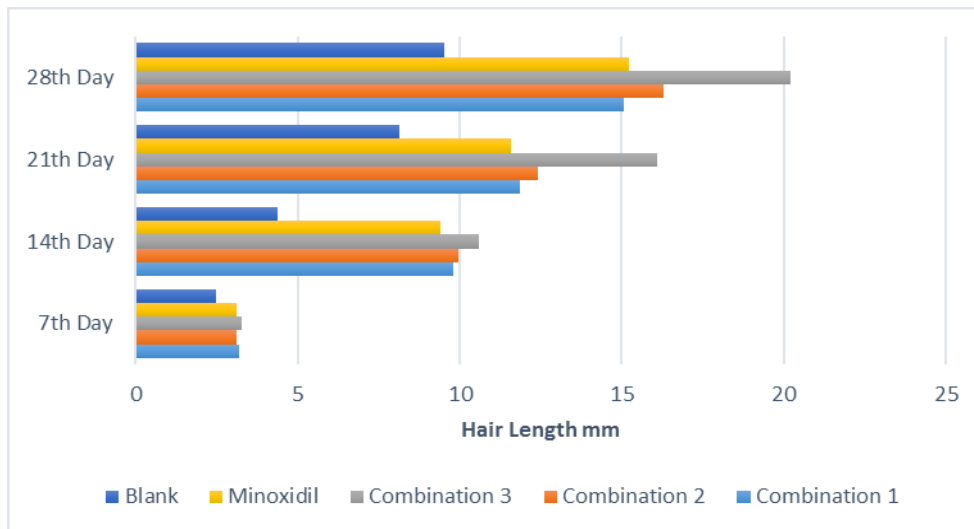


Fig.1 Graph of rabbit hair growth rate on days 7<sup>th</sup> to 28<sup>th</sup>

Table 4 Result on Hair Length of white rabbits in hair growth activity

No	Sample	Hair Length on day 28 <sup>th</sup> mm (mean ± s.d.)
1	Combination 1	15.06±0.05
2	Combination 2	16.28±0.10
3	Combination 3	20.22±0.19
4	Minoxidil	15.24±0.15
5	Blank	9.53±0.04

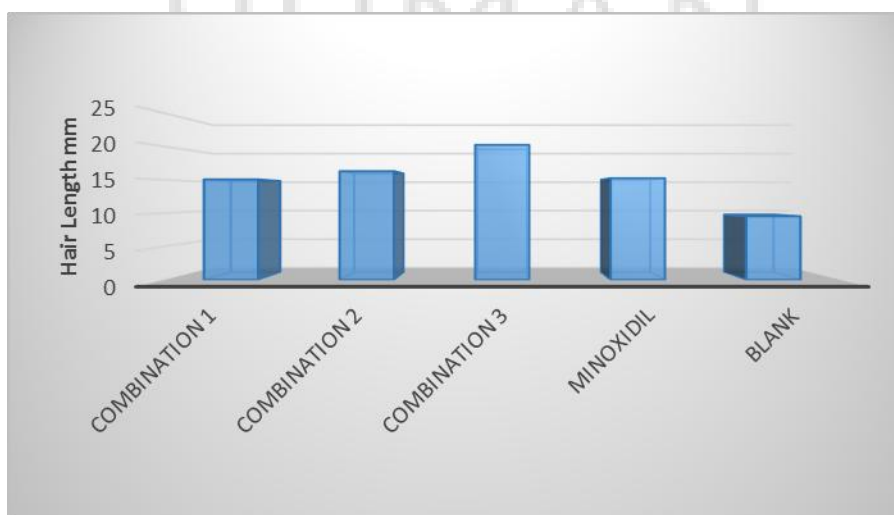
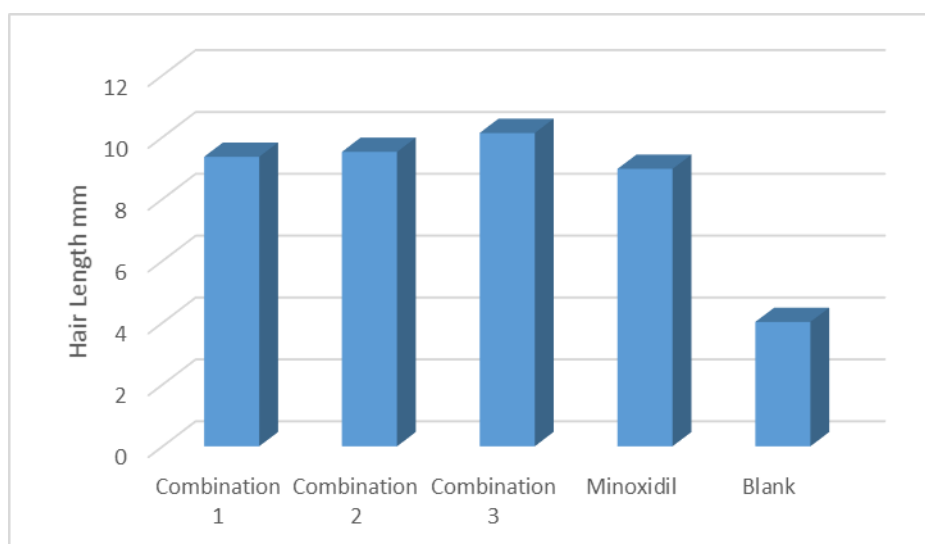


Fig.2 Average chart hair length on day 28<sup>th</sup>

**Table 5 Average Growth Daily (AGD) gain 3 rabbits after administration sample for 21 days**

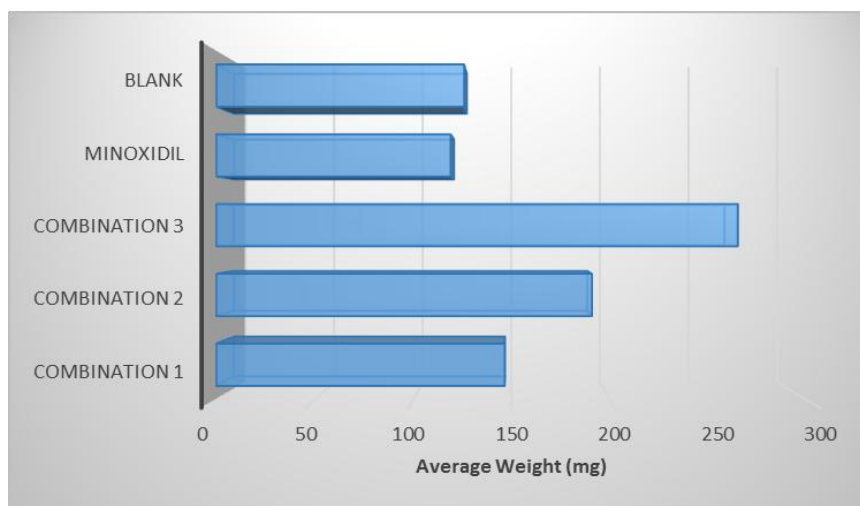
No	Sample	AGD
1	Combination 1	9.36
2	Combination 2	9.52
3	Combination 3	10.13
4	Minoxidil	8.97
5	Blank	4.02



**Fig. 3 Graph Average Growth Daily (AGD) after administration for 21 days**

**Table 6 Average hair weight 3 rabbits after administration sample for 28 days**

No	Sample	Average Weight (mg)
1	Combination 1	146.70
2	Combination 2	191.05
3	Combination 3	265.18
4	Minoxidil	119.15
5	Blank	126.05



**Fig. 4 Graph average hair weight rabbits after administration for 28 days**

The irritation tests were performed on all the samples and the results in table 3 showed all samples that used in the study did not cause erythema and edema on skin of rabbit. This indicates that the prepared formulation was non-irritant on skin of rabbits. The results hair growth was seen from the first week until 4<sup>th</sup> week, data showed the average length hair normal controls, extracts combination 1, extracts combination 2, extracts combination 3, standard and blank, respectively, are  $15.06 \pm 0.05$  mm ,  $16.28 \pm 0.10$  mm,  $20.22 \pm 0.19$  mm,  $15.24 \pm 0.15$  mm and  $9.53 \pm 0.04$  mm. Based on the results, the best combination was combination 3.

The ability as a hair growth caused compound saponins, phenols and flavonoids. Saponins have the ability to form foam, which means it can clean the skin of impurities, resulting in an increase in peripheral blood circulation thereby increasing hair growth. Likewise, the derivatives of phenol which has activities keratolytic, disinfectants, as well as flavonoids have activity as bactericidal and anti-viral which can suppress the growth of bacteria and viruses, so it can accelerate hair growth and prevent hair loss.

Average length of hair on 28<sup>th</sup> day showed the data normally distributed so homogeneous and direct statistical analysis using ANOVA test. The test results showed that there were significant differences ( $p < 0.05$ ) in blank compared with a combination 1, 2, and 3, this indicates that hair growth is statistically significant at the third combination on day 28 when compared with normal controls. Statistical data shows significant differences ( $p < 0.05$ ) in the average length of hair between the combination and minoxidil on the 28<sup>th</sup> day. This shows that all the combinations on the 28<sup>th</sup> day have activity as hair growth, but its effectiveness is

not the same as compared to the minoxidil. Comparisons between each combination 1, 2 and 3, said there are significant differences ( $p > 0.05$ ) between the combinations.

## CONCLUSION

In the present study, all samples do not cause irritation on skin of rabbits. From the observation of 28 days, the combination of the ethanol extract of basil and noni leaves has hair growth activity; with the best combination of a combination of 3 which had an average hair length of the total of 20.22 mm and average hair weight 265.18 mg. It may be due to presence of volatile oil in basil extract; saponins, phenols and flavonoids, in both extracts.

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