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Research Article

IN VIVO HAIR GROWTH STIMULATING ACTIVITY OF ETHANOL EXTRACT AND ITS FRACTIONS FROM RAMPAI LAMPUNG (*LYCOPERSICON ESCULENTUM* Mill.) LEAVES

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ABSTRACT

Hair loss is a hair problem that often occurs in every individual. Side effects caused by the use of synthetic drugs cause herbal remedies to be an option in overcoming hair loss and to stimulate hair growth. Rampai leaves (*Lycopersicon esculentum* Mill.) has traditionally been used to stimulate hair growth in the Lampung tribe. This study aim was to examine the activity of hair growth stimulation effects of ethanol extract and fraction (n-hexane, ethyl acetate, and water) from rampai leaves. The activity test of hair growth stimulation effect was carried out topically on New Zealand White (NZW) male rabbits for 21 days with the modified Tanaka method and using 2% minoxidil as a positive control The analysis results were evaluated statistically. The test results of ethanol extract of rampai leaves showed the most significant hair growth stimulation activity at 10% concentration, followed by a concentration of 20% and 5%. In testing the activity of fraction, n-hexane fraction significantly showed better hair growth activity compared to ethyl acetate, water fractions, and positive control. This showed that rampai leaves had vity of hair growth stimulation effects could be used as anti-alopecia treatment. Further experiments are needed to find the chemical compound content that is responsible for the nature of hair growth.

Keywords: rampai leaves, *Lycopersicon esculentum*, medicinal plants, hair growth, ethanol extract

INTRODUCTION

Hair loss that can lead to baldness is one of the problems that most people fear [1]. Hair care with herbal ingredients has long been known by tribes in Indonesia such as the Wajak tribe, Baduy tribe, Javanese tribe, Batak tribe, Malay tribe, Betawi tribe, Acehnese tribe, Toraja tribe, Rejang tribe, Balinese tribe, Dayak tribe, and tribe Papua. Based on the general knowledge that has long been developed in the community and the slogan "*back to nature*" the researchers were moved to utilize herbal ingredients, not only in the field of medicine such as synthetic ingredients such as minoxidil but also in the field of cosmetics [2].

Lampung tribe uses Coconut (*Cocos nucifera*) and Rampai (Indonesian) (*Lycopersicon esculentum* Mill.) fruit for hair treatment and fertilization. Coconut (*Cocos nucifera*) is used as shampoo, the fruit is first shredded, and the juice is used for shampooing while rampai leaves for pounding the hair, applied to the scalp twice a day [3]. Rampai leaves have a toxic glycoalkaloid content which when consumed by humans in large quantities can lead to difficulty breathing, nausea, vomiting so that people are reluctant to use it [4]. In addition to the mineral content, it also contains peroxidase enzymes obtained from the fruit, leaves, and stems of the stem tissue [5]. Rampai leaves contain peroxidase and together with H_2O_2, the compound can be converted into hypothiocyanite ($OSCN^-$) which has antimicrobial activity [6].

In order to discover new drugs as an alternative treatment for hair loss, an ethnopharmaceutical approach can be used to determine the types of plants that have high potential and how to use them based on an empirical knowledge that is believed by the people in an area. Based on the description above, in this study an activity test of hair growth stimulation effect from *Lycopersicum esculentum* Mill. extract and fraction of natural extracts substituted for minoxidil were carried out.

MATERIALS AND METHODS

Materials Plant

The material used in the form of rampai leaves were obtained from cultivation plantations in Gisting District, Tanggamus Regency, Lampung Province. Solvents (ethanol, ethyl acetate, n-hexane), arboxymethyl cellulose (CMC) and minoxidil 2% as the positive control were purchased from a local supplier. The chemicals used were Mayer, Dragendorff reagents, 10% vaniline in concentrated sulfuric acid, magnesium powder, 10% sulfuric acid in 10% ethanol, Lieberman-Burchard reagent, KOH, and 95% Ethanol. If not stated otherwise, all reagents were analytical grade.

Equipments: Moisture analyzer, Rotary Evaporator (Buchi®), macerator, Digital Caliper Stainless hardened.

Animal Test

The test animal used male rabbits of New Zealand White (NZW) strain (*Oryctolagus cuniculus* (4-5 months, with weights ranging from 2-4 kg) were obtained from Livestock Research Center (Balitnak, Bogor, Indonesia). Before being used for testing, rabbits were adapted and acclimatized first for 1 week to observe their health. Rabbits were fed a special feed with adequate

nutrition. Ethical approval for testing with animals was obtained from the Research Ethics Committee, Universitas Padjadjaran (No.351/UN6.KEP/EC/2018).

Methods

The research method that was used in this research was firstly collection and processing of materials and determination of rampai plants, phytochemical screening of leaves and viscous rampai leaves extract, based on modifications of the Farnsworth method [7]; The extraction of rampai leaves (*Lycopersicon esculentum* Mill.) used 95% ethanol solvent by maceration method. The maceration process was based on modifications of Mustarichie *et al.* Method [8]. Examinations of ethanolic extract parameters of rampai (*Lycopersicon esculentum* Mill.) Based on Indonesia Pharmacopoeia IV [9, 10]; Fractionation of ethanol extract of rampai leaves was done by liquid-liquid extraction method (LLC). Modifications of the Mustarichie *et al*. Method [11] were applied and testing of hair growth stimulation activity of ethanolic extract and its fractions on male rabbit test animal using Tanaka *et al*.[12]

Hair Growth Stimulating Activity Test

Animal Preparation Tests and Hair Growth Stimulating Activity Tests

Male rabbits must be quarantined for 7 days before being treated so that they are accustomed to the environment when treated, then rabbits are weighed. Activity testing method used is Tanaka (1980) method which has been modified where on the 6th day of quarantine, the back of the rabbit is shaved to baldness. After that, 70% ethanol antiseptic was applied to the rabbit's back. The rabbit is rested for 24 hours. The bald rabbit back is divided into 7 boxes (2 x 2 cm^{-2}) with a distance of 1 cm each box. Following is the distribution of treatment for each tested solution is (1) normal control (not treated), (2) negative control (CMC carrier solution), (3) positive control (minoxidil 2%), (4) extract 5%, (5) extract 20%, (6) and extract 10%. The treatment was carried out twice a day in the morning and evening as much as 0.5 ml. The parameters measured for the study were the length of rabbit hair which was measured every 3 days for 21 days of treatment, where the first day of application was considered the 0th day. Measurements are made by taking the 6 longest strands of hair from each section. The hair is straightened on a flat plane, then affixed to the field with tape, then measured using a caliper.

Test the fraction of the distribution of treatment for each tested solution was as follows (1) normal control (not treated), (2) negative control (CMC carrier solution), (3) positive control (minoxidil 2%), (4) n-hexane fraction, (5) ethyl acetate fraction, (6) water fraction, and (7) ethanol extract 10%.

Statistical Analysis

Data obtained in the form of hair length. All data are expressed as means ± SD was analyzed by statistical Analysis of variance (ANOVA) and Tukey HSD post-hoc tests were performed for statistical analysis of data using IBM© SPSS© Statistics version 22.00. at the level of $\alpha = 0.05$ (if it met the prerequisites for normality and homogeneity, the significance value was <0.05)

RESULTS

Plant determination: the sample used was *Lycopersicon esculentum* Mill.

Table 1: Phytochemical screening of ethanolic extract from *L. esculentum* leaves

Secondary metabolites	Ethanol extract
Alkaloids	+
Flavonoids	+
Polyphenolics	+
Tannins	-
Quinone	-
Monoterpenoids and Sesquiterpenoids	+
Steroids and Triterpenoids	+

Notes: +: detected, -: not detected

Table 2: The pharmacognostic parameters of *L. esculentum* leave

Parameters	Plants	Extract
	(%)	
Loss on drying	8.24	19.85
Ethanol soluble extractive	2.51	15.36
Water-soluble extractive	2.61	10.63
Water content	13.58	23.19
Total ash content	23.27	32.70
Acid-insoluble ash	3.04	0.86
Water soluble ash	8.24	19.85

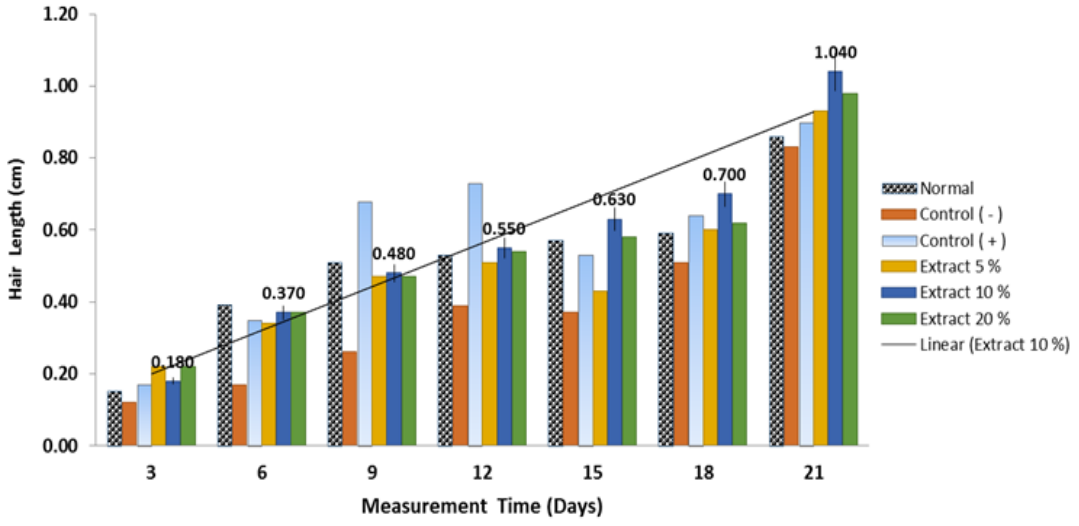

Fig. 1: Result of activity test ethanolic extract *L. esculentum* leaves

Table 3: Results of the hair length measurement activity test ethanolic extract of *L.esculentum* leaves

Treatment	Measurement of rabbit hair on the day - (cm)						
	3	6	9	12	15	18	21
normal control	0.14	0.37	0.49	0.44	0.49	0.54	1.02
	0.12	0.41	0.47	0.46	0.57	0.62	0.69
	0.15	0.39	0.56	0.73	0.65	0.57	0.86
	0.18	0.38	0.50	0.48	0.56	0.63	0.85
Mean ± SD	**0.15 ± 0.02**	**0.39 ± 0.02**	**0.51 ± 0.04**	**0.53 ± 0.14**	**0.57 ± 0.07**	**0.59 ± 0.04**	**0.86 ± 0.13**
negative control	0.10	0.14	0.20	0.24	0.35	0.49	0.88
	0.09	0.12	0.28	0.47	0.32	0.51	0.92
	0.13	0.26	0.35	0.56	0.46	0.56	0.75
	0.15	0.16	0.21	0.30	0.35	0.48	0.77
Mean ± SD	**0.12 ± 0.03**	**0.17 ± 0.06**	**0.26 ± 0.07**	**0.39 ± 0.15**	**0.37 ± 0.06**	**0.51 ± 0.04**	**0.83 ± 0.08**
positive control	0.18	0.32	0.60	0.73	0.43	0.81	1.05
	0.21	0.37	0.64	0.71	0.36	0.61	0.96
	0.17	0.35	0.68	0.75	0.65	0.71	0.87
	0.12	0.36	0.79	0.73	0.67	0.42	0.70
Mean ± SD	**0.17 ± 0.04**	**0.35 ± 0.02**	**0.68 ± 0.08**	**0.73 ± 0.02**	**0.53 ± 0.16**	**0.64 ± 0.17**	**0.90 ± 0.15**
extract 5 %	0.18	0.31	0.26	0.22	0.50	0.67	1.00
	0.19	0.29	0.42	0.46	0.37	0.65	1.06
	0.23	0.42	0.61	0.60	0.61	0.71	0.99
	0.27	0.34	0.60	0.77	0.23	0.35	0.67
Mean ± SD	**0.22 ± 0.04**	**0.34 ± 0.06**	**0.47 ± 0.17**	**0.51 ± 0.23**	**0.43 ± 0.16**	**0.60 ± 0.17**	**0.93 ± 0.18**
extract 10 %	0.17	0.28	0.35	0.46	0.52	0.60	0.97
	0.15	0.36	0.42	0.55	0.63	0.68	1.01
	0.16	0.41	0.61	0.64	0.71	0.79	1.10
	0.22	0.44	0.54	0.56	0.67	0.71	1.06
Mean ± SD	**0.18 ± 0.03**	**0.37 ± 0.07**	**0.48 ± 0.12**	**0.55 ± 0.07**	**0.63 ± 0.08**	**0.70 ± 0.08**	**1.04 ± 0.06**
extract 20 %	0.16	0.36	0.43	0.51	0.60	0.63	0.93
	0.14	0.27	0.45	0.55	0.51	0.58	0.86
	0.26	0.46	0.58	0.62	0.69	0.72	1.12
	0.31	0.38	0.42	0.48	0.52	0.55	1.01
Mean ± SD	**0.22 ± 0.08**	**0.37 ± 0.08**	**0.47 ± 0.07**	**0.54 ± 0.06**	**0.58 ± 0.08**	**0.62 ± 0.07**	**0.98 ± 0.11**

SD = Standard Deviation

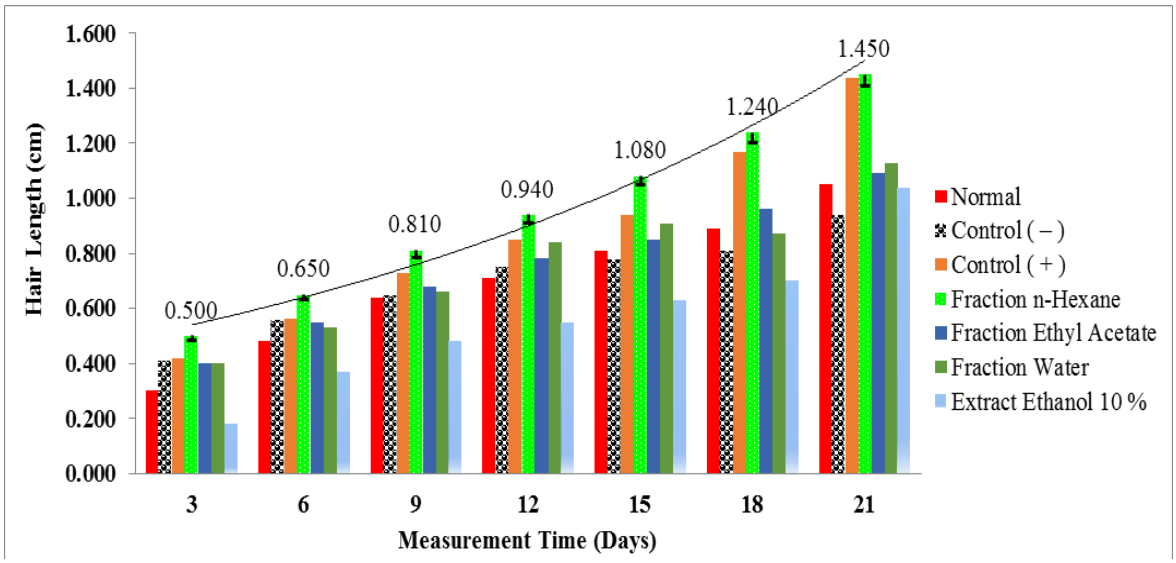

Fig. 2: Results of the activity test of hair length measurement of n-hexane fraction, ethyl acetate fraction, water fraction and ethanolic extract 10% *L. esculentum* leaves

DISCUSSION

Collecting and Plant Determination

Rampai leaves (*Lycopersicon Esculentum* Mill.) was collected from cultivation plantations in Gisting District, Tanggamus Regency, Lampung Province. Determination of rampai plants was carried out at the Herbarium Bogoriense, Field of Botany, Research Center of Biology, Indonesian Institute of Science (LIPI) Bogor. Determination results showed that the samples used were the *Solanaceae*, the *Lycopersicon* genus, and the *Lycopersicon esculentum* Mill. species. Leaves that had been collected and dried, sorted to separate the other undesirable parts of the plant and other impurities left behind in dried simplicial [13]. Simplicia was further reduced by using scissors and blenders to increase the surface area of the simplicia so as to facilitate the extraction process because the more surface area allowed the solvent to reach cells [14,15]. The small size of the simplicia particles

will expand the contact of the simplicia with the solvent, resulting in more secondary metabolites being attracted [16].

Extraction and Fractionation

Extraction of 2,168.2 g of dried plants, by maceration for 3 x 24 hours using 95% ethanol produced a yield of 280 g of thick extract (12.91%). A total of 156.6 g of thick extracts were taken for fractionation. In fractionation using n-hexane, ethyl acetate, and water with a ratio of 1: 1 each obtained three fractions with weighing each n-hexane fraction (29.4 g), ethyl acetate fraction (11.2 g), and water fraction (18.7 g). Ethanol was chosen as a solvent in the extraction process because it had high solubility, most of the secondary metabolites were insoluble, non-toxic, and inert so they do not interfere with other components. The use of ethanol as a solvent could prevent the growth of fungi and bacteria in the extract, so as to minimize the occurrence of contamination in the extract. The low ethanol boiling point facilitates the evaporation process in the extraction process and extracted simplicia with less heat [17,18].

Phytochemical Screening

The results of a phytochemical screening test for ethanolic extract rampai leaves (*Lycopersicon esculentum* Mill.) showed the content of alkaloids, flavonoids, saponins, quinones, monoterpenoids, sesquiterpenoids, steroids and triterpenoids. The results of phytochemical screening of ethanolic extract rampai leaves can be seen in Table 1.

Rafiqkha [19] on their study on pharmacognostic and phytochemical Investigation of *Lycopersicon esculentum* (Tomato) flower extracts revealed that the flowers of Lycopersicon esculentum presence of alkaloids, flavonoids, terpenoids, phenolic compounds, sterols, carbohydrates, glycosides, and tannins. Trace amounts of saponin and tannin (Tannin Acid Equivalent) were found in the cherry tomato fruits from Ikere –Ekiti, Nigeria [20]. Erturk *et.al* [21.] from Turkey in their phytochemical analysis of the cherry tomato fruits revealed the presence of carbohydrates, saponins, flavonoids, glycosides, tannins, phenols and alkaloids in the extracts. The difference in the results of phytochemical screening, was probably due to differences in plant origin.

Pharmacognostic Parameters

Pharmacognostic parameters were determined to refer to Indonesian Pharmacopoeia IV [21] and Materia Medika Indonesia [23]. Table 2 showed the result of a pharmacognostic parameter of rampai leaves extract. Determination of this extract parameter aimed to determine the standardization of rampai leaves extract parameters. The water content of the extract was high because the extract was hygroscopic so it made it easy to bind water around it. The content of water-soluble extract and the extract obtained was quite large. The content of this essence was related to the determination of the dosage calculation of an herbal preparation. Pharmacognostic parameters of Pharmacopoeia IV [21] and Materia Medika Indonesia [23] slightly different from WHO guidelines on which the WHO guidelines mentioned for the safety of herbal medicines [26]. Regarding ash content, it aimed to give a good mineral description derived from the initial process until the formation of extract from both inside and outside contained in the extract. There were two kinds of mineral salts in a material that was organic salts, such as salts of oxalic acid, acetate, malate, etc. and inorganic salts, such as phosphates, chlorides, phosphates, alkali metals, and nitrate sulfates. The low acid soluble ash content indicated a low impurity content such as sand and silicate.

Determination of moisture content was done by distillation method by using a solvent type which could not mix with water (immiscible). The solvent used was toluene, this solvent had a boiling point higher than that of 100.6 °C and a lower specific gravity of 0.866. The smaller the water content in the extract would be less likely to be contaminated by bacteria and fungi. Our water content either in plat or extract met the literature requirements for viscous extracts of 5-30% [25].

Hair growth stimulating activity test of ethanol extract

The test of hair growth stimulator of rampai leaves extract was performed on male rabbit test animals based on a modification of Mustarichie *et al*. and Tanaka *et al*. methods [11,12]. The test was carried out with the following steps: Preparation of testing of rampai leaves extract, preparation of test animal. In this study, male rabbits were used as test animals because male rabbits were hormonally more stable than female rabbits. The selected male rabbit was a healthy, healthy rabbit aged 4–5 months, and weight 2-2.5 kg because it had excellent and perfect physiological function. The study had obtained approval from the Research Ethics Committee to protect the rights and welfare of test animals. Before the test, rabbits acclimatized and allowed to adapt to the condition of the cage so that the rabbit was not stressed. On the 7th day of acclimatization, the rabbit's back was shaved clean. The tested solution on the rabbit's back divided into seventh parts consisted of part 1: normal control (not treated), part 2: negative control (CMC carrier solution), part 3: positive control (minoxidil 2%), part 4: extract 5%, part 5: extract 20%, and part 6: extract 10%

The measurement of rabbit hair growth was based method [12] which was carried out for 21 days with the taking time period every three times a day. Measurements were made by taking the three longest strands of hair from each test material. Then it was calculated using the caliper run and calculated the average hair length of each test material. Taking 3 strands of each test material was done so that the results obtained would represent the total hair growth because each rabbit's hair had a different length. Besides that, by taking 3 pieces of each box (test material) it was expected that the loss due to extraction would be avoided.

The Na CMC solution as a negative control functioned to know that the base used (Na CMC) in the manufacture of test solution did not give effect to the animal test and did not have the effect of stimulating hair growth. The parameter used in this test was the length of the rabbit's hair. The test solution was given to the rabbit's back at a different location on each animal to reduce the measurement bias. The hair length of each rabbit of each part was averaged. The results of measurement of rabbit hair length on a test of rampai leave ethanolic extract can be seen in Table 3.

From Table 3, after 21 days of testing, among the test group, rampai leaves extract at 10% concentration showed the highest hair growth result that was 1.04 cm, while the ethanol extract test group of rampai leaves at concentration 5% showed the most hair growth results in low, i.e. 0.93 cm.

Fig. 1 showed that the growth of rabbit hair length at normal control and the negative control was not too fast compared with positive control (2% minoxidil) and test group. The ethanol extract test group leaves 10% rampai concentration has the highest average hair length at 1.04 cm. The ethanolic extract of rampai leaves at concentrations of 5%, and 20% had shown better hair growth results than negative controls and normal controls. The following test for hair growth stimulant activity at rampai leaves fraction was done at 10% concentration because, at this

concentration, ethanolic extract concentration of rampai leaves was not too small and had shown good hair growth activity.

Hair growth stimulating activity test of fraction ethanolic extract

Testing of hair growth stimulating activity for fractions was carried out using the same test method with 95% ethanol extract testing using the modified method Tanaka *et al*. [12] with the following treatments: part 1: normal control (not treated), part 2: negative control (CMC carrier solution), part 3: positive control (minoxidil 2%), part 4: n-hexane n, part 5: ethyl acetate fraction, part 6: water fraction, and part 7: ethanolic extract 10%. The result of the hair length measurement activity can be seen in Fig. 2.

In the graph of Fig.2, it could be seen that the fraction that showed the highest activity was the n-hexane fraction fraction after n-hexane was the water fraction then ethyl acetate fraction. All the rampai leaves fractions, however, had a better hair growth stimulant activity than positive control (2% minoxidil). In the normal control group and negative control, rabbit hair growth showed the same results. Hayati [27] showed in the study of the stimulating activity of hair growth from the ethanol extract of *Malvaviscus arboreus* leaf showed that the n-hexane fraction gave better hair stimulating activity than the other fractions studied. So far there has not been any publication regarding the efficacy of in-vivo hair growth stimulating the activity of ethanol extract and its fractions from Rampai Lampung Leaves (*Lycopersicon esculentum* Mill.)

Data Analysis

Data from rabbit hair length measurements were tested for normality and homogeneity first. Normality test was carried out using the Shapiro-Wilk method [28], while homogeneity was tested using the Levene Statistic method [29]. The results of testing normality and homogeneity of data showed a significance >0.05 so that it can be stated that the data were normally distributed and homogeneous. Furthermore, the data were analyzed using ANOVA, the results of the analysis showed a significance <0.05 so it was stated that there were significant differences in each treatment group. After that, further tests were conducted to see the extent of the differences between treatment groups. Further testing is done by the Least Significant Difference (LSD) method. Based on the analysis of the LSD method, the n-hexane fraction of rampai leaves had a significance <0.05 which showed that there were significant differences between the n-hexane fraction with negative, normal and positive controls so that it was stated that the n-hexane fraction of rampai leaves showed activity hair growth stimulants and activities were better than positive controls (2% minoxidil).

CONCLUSION

It was found that ethanolic extract rampai leaves (*Lycopersicum esculentum* Mill.) had activity in stimulating hair growth from 10% w/v ethanolic extract concentration and best at 15% w/v concentration. The n-hexane fraction, ethyl acetate fraction, and water fraction had a hair growth stimulant activity. It was found that the fraction of rampai leaves which gave the best activity in stimulating hair growth was n-hexane fraction. It is recommended that there is further research in the form of structural elucidation to know the chemical content responsible for this hair growth activity.

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