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POTENTIAL OF HAIR TONIC SARANG BANUA (*Clerodendrum fragrans*) LEAF EXTRACTS AS ANTI ALOPECIA *IN VIVO*

Potensi Hair Tonic Ekstrak Daun Sarang Banua (*Clerodendrum fragrans*) sebagai Anti *Alopecia* Secara *In Vivo*

Zhafira Dwika Ananda[1], Rahma Clarisa[1], Ruth Yohana Saragih[1], Nadia Givani Br Hotang[1], Gusti Ayu Lestari[2], Yesica Marcelina Romauli Sinaga[3], Murniaty Simorangkir[1*]

[1] Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Negeri Medan, Medan, Sumatera Utara, Indonesia.

[2]Department of Biology, Faculty of Mathematics and Natural Science, Universitas Negeri Medan, Medan, Sumatera Utara, Indonesia.

[3]Department of Food Science and Technology, Faculty of Food Technology and Agroindustry, Universitas Mataram, Mataram, Nusa Tenggara Barat, Indonesia.

*Email: murniatysimorangkir@unimed.ac.id

ABSTRACT

Alopecia is a condition where head hair is missing or not growing which can occur as a result of aging, malnutrition, hormonal imbalance. Hair tonic is a liquid preparation that is used to grow and fertilize hair. Hair tonic preparations as hair growth require high levels of antioxidants. Sarang Banua (Clerodendrum fragrans) contains has high antioxidant. This research aims to determine the potential of hair tonic sarang banua leaf extract as an anti-alopecia in vivo. The research method is in vivo with white wistar rats (Rattus norvegicus). This research was an experimental LSD with 5 groups, C– (no extract), C+ (minoxidil 2%), F1 (5% extract), F2 (7.5% extract), and F3 (10% extract) carried out 3 treatments. The treatment is carried out every day and applied to mice that experience stress and natural baldness. The ANOVA test results showed that F1 (5% extract) had no significant difference from the C+ (2% minoxidil).

Keywords: *anti alopecia, hair tonic, in vivo, Rattus norvegicus, sarang banua (Clerodendrum fragrans).*

ABSTRAK

Alopecia merupakan suatu keadaan hilang atau tidak tumbuhnya rambut kepala yang dapat terjadi akibat dari penuaan, kekurangan gizi, ketidakseimbangan hormon. Hair tonic merupakan sediaan cair yang digunakan sebagai penumbuh dan penyubur rambut. Sediaan hair tonic sebagai penumbuh rambut membutuhkan antioksidan yang tinggi. Sarang banua (*Clerodendrum fragrans*) memiliki antioksidan yang tinggi. Riset ini bertujuan untuk mengetahui potensi hair tonic ekstrak daun sarang banua sebagai anti alopecia secara in vivo. Metode riset dilakukan secara in vivo pada tikus putih wistar (*Rattus norvegicus*). Penelitian ini merupakan penelitian eksperimental Rancangan Acak Lengkap (RAL) dengan 5 kelompok, K– (tanpa ekstrak), K+ (minoxidil 2%), F1 (5% ekstrak), F2 (7,5% ekstrak), dan F3 (10% ekstrak) dilakukan 3 perlakuan. Perlakuan dilakukan setiap hari dioleskan ke tikus yang mengalami stress dan kebotakan alami. Hasil uji ANOVA menunjukkan bahwa F1 (ekstrak 5%) tidak ada perbedaan signifikan dengan K+ (minoxidil 2%).

Kata Kunci: anti alopecia, hair tonic, in vivo, Rattus norvegicus, sarang banua (Clerodendrum fragrans).

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INTRODUCTION

Having healthy and soft hair is everyone's dream. Soft hair is believed to be one of the requirements for an attractive appearance. It is not surprising that many women flock to salons to care for and beautify their hair and are even willing to dig deep into their pockets for the treatment (Armandari et al. 2021). Hair is a crown for everyone because apart from its function of providing warmth and protection, hair is also for beauty and supporting appearance. Healthy hair has the characteristics of being thick, black, shiny, doesn't tangle and doesn't fall out which is what everyone needs. Hair is found in almost all parts of the body and has various functions, including aesthetic functions for humans. One problem that still causes a person's self-confidence to decrease in activities is hair loss. Hair loss (*hair loss*) occurs in many people, so it can reduce the function of cosmetics and their protection of the body and head from the environment. This is not life threatening, but it affects self-confidence and can even be a psychological stressor. Hair loss is a disorder in which the amount of hair is smaller or sheds more than normal, with or without visible thinning. The normal number of hair follicles on the head is around 100,000, and it is called an abnormality if the number only reaches 50%, which means around 50,000 strands. Normally 80-120 strands of head hair are shed per day (Eryaputri et al. 2023).

Alopecia is a condition of loss or non-growth of head hair that can occur in both women and men (Nasution et al. 2020). Alopecia 6.8 million people in the United States and 147 million worldwide have or will suffer from alopecia areata at some point in their lives (Santi and Jaya, 2020). Reason alopecia including aging, malnutrition, hormonal imbalance, disease and stress (Fakhrizal and Saputra, 2020). Alopecia areata is an immune-mediated condition that causes non-scarring alopecia on the scalp and other hair-covered areas of the body. This disease affects up to 2% of the global population. This disease can attack all ages, but its prevalence appears to be higher in children than adults (1.92%, 1.47%). A greater incidence is reported in women than in men, especially in countries with the largest populations. patients with late-onset disease, defined as over 50 years of age (Sibbald, 2023).

Hair tonic is a liquid preparation used to grow and nourish hair, usually derived from plant extracts and a mixture of other chemicals. Hair tonic is designed to strengthen hair roots and maintain a healthy scalp so hair can grow. Hair tonic hair has a mechanism for effectively strengthening hair by stimulating the growth of hair roots which contain melanocytes which color hair and synthesize hard keratin. The formulation of hair nutrition preparations for hair growth includes solvents/carriers, preservatives, penetration enhancers, humectants, and antioxidants (Sahira and Darusman, 2021). There are many tonics on the market, both chemical and herbal ingredients. The use of chemicals in cosmetics is considered unsafe because of the potential side effects of long-term use. Hair tonic was chosen because it is in the form of a solution, easy to apply, not sticky like semi-solid formulations, and does not leave crusts that cause dandruff (Rahmi et al. 2021).

Minoxidil was introduced as an antihypertensive drug and the discovery of its common side effect, hypertrichosis, led to the development of topical formulations to promote hair growth. Until recently, topical minoxidil was the mainstay treatment for alopecia and was used as an off-label treatment for other hair loss conditions. Some of the side effects that have been reported when using minoxidil include skin irritation such as erythema, discomfort, and a burning sensation as well as allergies to the substance. Conventional drugs such as minoxidil and finasteride are widely used for treatment. However, some side effects have been reported such as contact dermatitis, burning sensation on the scalp, ejaculation disorders, and decreased libido (Yuda et al. 2023).

Apart from treatment with chemical drugs, currently therapies have been developed using natural ingredients. Therapy using herbal ingredients generally has several advantages, including relatively cheap

prices and widely available raw materials in Indonesia (Febriani et al. 2016). In previous research, hair tonic was made using local plants, namely 2.5% and 5% ethanol extract of hibiscus leaves, which had hair growth-stimulating activity that was equivalent to hair tonic containing 2% minoxidil, while hair tonic preparations containing ethanol extract of flower leaves shoes at 10% have significantly better activity compared to hair tonic containing 2% minoxidil (Febriani et al. 2016). Hair tonic related to anti-alopecia must have several activities to provide maximum care to the hair and scalp. This activity consists of antioxidant and antibacterial activity (Anwar and Darusman, 2022).

Sarang Banua are often found in the Simalungun and North Tapanuli areas, North Sumatra (Figure 1). As a result of plant determination, the *sarang banua* (local name) is a type *Clerodendrum fragrans* Vent Willd, belongs to the Verbenaceae family (Simorangkir et al. 2019b). Simorangkir et al. (2018) reported that extract of *sarang banua* leaves contains secondary metabolites of alkaloids, steroids and flavonoids. The research results of Simorangkir et al. (2019a) showed that *sarang banua* leaf extract has high antioxidant activity. Ethanol extract and ethyl acetate extract of *sarang banua* leaves have very high antioxidant activity with LC_{50} values of 22.37 ppm and 27.26 ppm close to the LC_{50} of vitamin C (20.18 ppm) as an antioxidant standard using the radical scavenging method. DPPH free.

Figure 1. Sarang Banua Plants (Sumber: Simorangkir et al., 2018)

Using hair tonic can be an alternative for maintaining healthy hair and helping hair grow. However, many hair tonics on the market still contain synthetic substances such as minoxidil. Minoxidil, commonly used as a topical medication on the scalp to reduce hair loss, has been reported to cause allergic reactions, itching and dermatitis. Efforts to reduce these side effects are by using natural ingredients as active substances in hair tonic. One natural ingredient that is useful for helping hair growth is flavonoids. Several types of plants have been scientifically proven to treat hair damage with their flavonoid content (Anwar and Darusman, 2022). Therefore, it is important to carry out more in-depth research on traditional medicinal plants that can be used to help the hair loss problem, both by inhibiting the process of hair loss and increasing hair growth. Based on the description above, the researchers choose *sarang banua* leaves as a research subject because *sarang banua* leaves contain high levels of antioxidants with several secondary metabolites, among them are flavonoids, which have the ability to stop free radicals and accelerate the growth of hair. Researchers were interested in finding out how well the active components in *sarang banua* work as antioxidants and anti-free radical agent to prevent hair loss (anti-alopecia).

MATERIALS AND METHODS

Place and time of research

This research was conducted out in the chemistry laboratory and animal test house, Faculty of Mathematics and Natural

Science, Universitas Negeri Medan. Activities were carried out from July to September in stages.

Materials

The main ingredient used was Sarang Banua leaves fresh taken from Simalungun, North Sumatra. The experimental animals were white Wistar rats (*Rattus norvegicus*) aged 2-3 months, standard feed, as well as distilled water, n-hexane, ethanol 96%, ethyl acetate, filter paper, and the formulation with glycerin that uses as a humectant, which means it attracts and retains moisture. When used on hair, it helps to seal in moisture and soften the hair shaft, propyl paraben is added as a preservative (antimicrobial) in hair tonic, methyl paraben is used in hair tonic as a preservative to prevent the growth of mold and bacteria, thus extending the product's shelf life, menthol in hair tonic can help with hair growth by stimulating blood flow, revitalizing hair follicles, soothing irritated scalps, and promoting overall hair growth. The tools used are glassware, glass funnels, stirring rods, spatulas, animal cages, animal drinkers, grinders, sieves, tools, rotary evaporator, hot plate, analytical balance, Buchner funnel, vacuum pump, pH meter, pycnometer, Ostwald viscometer, thermometer, razor, scissors, vernier caliper.

Methods

1. Preparation of Sample and Leaf Extract of Sarang Banua

4.5 kg of fresh *sarang banua* leaves were washed, drained, dried and protected from direct sunlight and mechanically ground into simplicia powder. *Sarang banua* leaf simplicia powder was macerated with a solvent with high polarity, namely n-hexane solvent, followed by ethyl acetate solvent and ending with ethanol solvent. *Sarang banua* leaf simplicia powder was macerated with n-hexane solvent, filtered and the filtrate obtained was concentrated in a vacuum rotary evaporator to obtain concentrated n-hexane extract. The pulp part was macerated again with ethyl acetate and ethanol solvents, with the same treatment. Each maceration process for each solvent lasts for 48 hours, with the solvent added three times (Simorangkir et al. 2018).

2. Preparation of Hair Tonic Preparation Formula from Sarang Banua Leaf Extract

Table 1 shows the materials that will be created by figuring out the formula composition.

Table 1. Hair Tonic Preparation Formula (Sumakno et al. 2021)

Materials	Amount (mL)			
	Negative Control	F1	F2	F3
Sarang Banua Leaf Extract	-	5	7,5	10
Ethanol 96%	15	15	15	15
Glycerin	30	30	30	30
Propyl Paraben	0,4	0,4	0,4	0,4
Methyl Paraben	0,3	0,3	0,3	0,3
Menthol	2	2	2	2
Aquadest	50	50	50	50

1. *In Vivo* Hair Growth Test

This research was subject to Health Research Ethics Approval issued by the Animal Research Ethics Committees/AREC) No. 0501/KEPH-FMIPA/2023 from Animal Research Ethics Committees, Faculty of Mathematics and Natural Science, Univesitas Sumatera Utara. The research was carried out experimentally with a Completely Randomized Design (CRD) with 5 treatments, namely C– (no extract), C+ (minoxidil 2%), F1 (5% extract), F2 (7.5% extract), and F3 (10% extract) for each treatment with repetition 3 times. The treatment was carried out every day in the amount of 1 mL which was applied to the backs of mice that were experiencing stress and natural baldness carried out aseptically. Hair length measurements were carried out every 3 days by measuring the length of the hair from the base of the hair to the tip of the hair

using a caliper. The measurements were carried out in triplicate (Mulyanti et al. 2019).

2. Data analysis

The *in vivo* test data obtained were analyzed statistically using Completely Randomized Design ANOVA (CRD) followed by the Least Significant Difference Test (LSD).

RESULTS AND DISCUSSION

1. *In Vivo* Test Results

This test is carried out by applying the preparation every day and measuring the hair growth of the test animal every 3 days in millimeters (mm) using a caliper. It can be seen in Table 2 that there was hair growth activity in the test animals in the positive control, negative control, formula 1, formula 2, to formula 3. From the treatments carried out, it can be seen that each formula has anti-alopecia activity. The mice used are mice that experience natural baldness, such as those suffering from alopecia, and each formula can grow hair in people with alopecia or baldness in an uneven area. However, it can be seen that formula 1 has activity that is not significantly different from the positive control formula. This can be seen from the following table.

Table 2. Hair Length Results (mm)

Day	C(+)			C(-)		
	1	2	3	1	2	3
3	4,33	4,21	4,27	1,09	1,25	1,17
6	7,88	7,55	7,71	1,98	1,87	1,93
9	10,07	10,05	10,06	3,44	3,54	3,49
12	14,98	14,24	14,61	3,90	3,94	3,92
15	17,72	17,45	17,59	4,71	4,54	4,63
Totals		17,59			4,63	

Day	F1			F2			F3		
	1	2	3	1	2	3	1	2	3
3	4,91	4,24	4,56	1,55	1,45	1,50	1,29	1,08	1,19
6	7,41	7,08	7,23	5,04	5,12	5,16	2,08	2,11	2,09
9	9,28	9,34	9,31	6,31	6,34	6,33	3,67	3,63	3,65
12	13,01	13,06	13,03	9,32	9,34	9,33	6,77	6,75	6,76
15	16,45	16,39	16,41	13,08	12,87	12,98	9,04	8,87	8,95
Totals		16,42			12,98			8,95	

The hair growth activity test for 15 days (2 weeks) shows that the hair tonic formula 1 preparation has an activity to stimulate hair length growth that is equivalent to minoxidil (positive control). Where formula one has hair length growth of 16.42 mm and the positive control is at 17.59 mm. Meanwhile, formula 3 with a mouse hair growth length of only 8.95 mm is a poor formula because it has significantly shorter hair length growth activity compared to the positive control which uses a commercial product containing the active ingredient minoxidil. From the table we can infer that the mouse hair growth length in Figure 2 that the negative control, namely without extract from *sarang banua* leaves, are very different from the hair growth results from the positive control, namely using minoxidil and formula 1 with 5% *sarang banua* leaf extract.

1. Data analysis

Data analysis using ANOVA aims to determine whether there are significant differences in the hair length of each group of white mice. With the Ho hypothesis, namely that there is no significant difference in the hair length of each group of mice and Ha, namely that there is a significant difference in the hair length of each group of mice. With a significant value of $\alpha = 0.05$. Where Ho is rejected if the significance value is $< \alpha$ and Ho is accepted if the significance value is $> \alpha$.

The results of analysis using ANOVA (Table 3) obtained a significant value of <0.05, it was concluded that Ho was rejected, Ha was accepted, namely that there were significant differences in the hair length of each group of mice. There was a significant difference from the ANOVA test, so it was continued with the Least Significant Difference Test (LSD) in hair length for each group of white mice (Table 4).

Table 3. ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	695.060	4	173.765	12.380	.000
Within Groups	982.525	70	14.036		
Total	1677.585	74			

The Least Significant Difference Test (LSD) aims to determine whether there are significant differences in hair length data for each group of white mice. With the Ho hypothesis, namely that there is no significant difference in the hair length data for each group of white mice. And Ha, that is, there is a significant difference in the hair length data for each group of white mice. With criteria where, α : 0.05.

Table 4. Least Significant Difference Test (LSD)

(I) Hair Tonic	(J) Hair Tonic	Difference Means (I-J)	Sig.
C+	C-	7.82133*	.000
	F1	.73400	.593
	F2	3.80000*	.007
	F3	6.31933*	.000
C-	C+	-7.82133*	.000
	F1	-7.08733*	.000
	F2	-4.02133*	.004
	F3	-1.50200	.276
F1	C+	-.73400	.593
	C-	7.08733*	.000
	F2	3.06600*	.028
	F3	5.58533*	.000
F2	C+	-3.80000*	.007
	C-	4.02133*	.004
	F1	-3.06600*	.028
	F3	2.51933	.070
F3	C+	-6.31933*	.000
	C-	1.50200	.276
	F1	-5.58533*	.000
	F2	-2.51933	.070

The results of the minoxidil group (positive control) and formula 1 research had a significant value of > 0.05, so Ho was accepted. So there was no significant difference in the hair length data of the minoxidil group (positive control) and formula 1 in white mice.

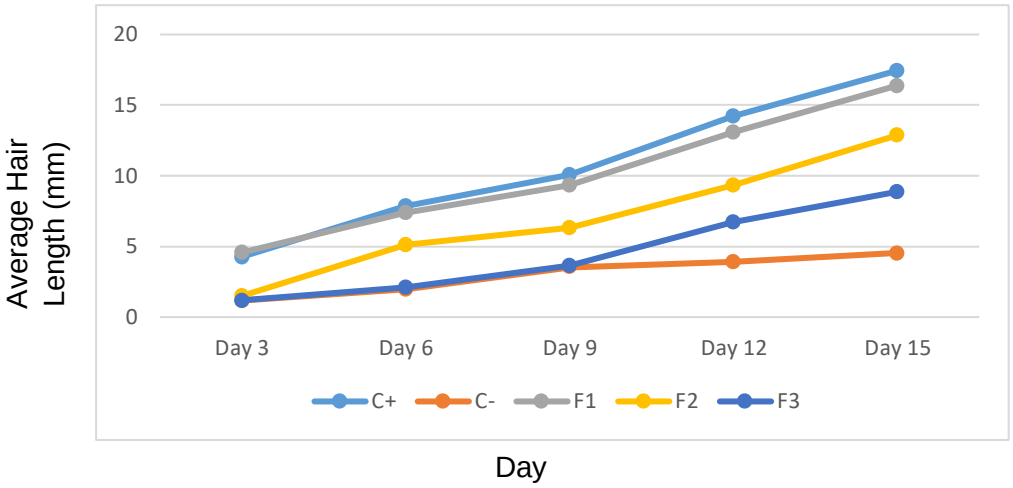

Figure 2. Hair Length Growth Chart

CONCLUSION

It can be concluded that the anti-alopecia activity of the Formula 1 preparation (*sarang banua* extract 5%), which was based on research on the formulation and anti-alopecia activity on hair growth of hair tonic, *sarang banua* with extract variations, namely 5%, 7.5%, and 10%, was not significantly different from the positive control group (minoxidil 2%). Formula 1 (5% *sarang banua* extract) is the optimal concentration compared to concentrations of 7.5% (F2) and 10% (F3).

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