Hair Growth Activity Test of White Ginger (*Zingiber officinale* Roscoe) Extract and Red Ginger (*Zingiber officinale* Rubra) Extract

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Abstract: Hair was protector against temperature and physical barrier. Hair loss was one of the most worrying. The objective of this research was conduct scientific tests on hair activity of white ginger extract and red ginger extract in the experimental animal.Extraction of white ginger and red ginger by soxhletation method. The obtained extract was formulated to simple gel with carbopol based gel. Experiments animals were male rats sprague dawley strain has been shaved and smeared. Treated with gel and observed hair growth time, hair length, and hair mass.The initial hair growth time for 2% minoxidil was on 4th day. White ginger extract gel was showed the same activity with 3% extract, but red ginger extract gel was showed the same activity with 1% extract. The final hair growth time for 2% minoxidil was on 9th day. White ginger extract gel did not showed the same activity with 3% extract, but red ginger extract gel was showed the same activity with 2% extract. White ginger extract has weakerhair growth activity than minoxidil, but red ginger extract has stronger hair growth activity than minoxidil.

1 INTRODUCTION

Hair is found in almost all parts of the body and has various functions, such as protection against ambient temperature and physical barrier between external air and skin, keep the body warmer and hair has its own aesthetic value for humans. One of the most worrying problems for everyone is hair loss that can result in baldness. Hair has different growth and fall periods on each strand. Although hair loss is a natural cycle of hair, sometimes the quantity and frequency of hair loss increase, causing baldness. This is caused by hormonal disorders, drug side effects, food intake, and stress (Guo and Katta, 2017).

Minoxidil, a drug of scientific origin was scientifically proved for the treatment of alopecia, but side effect associated with this drug has limited its pharmacological benefits hence the drug of plant origin is necessary to replace the synthetic one (Rathi et al., 2017). India is a repository of medicinal plants According to the researchers, innovation found an effective formula can solve the problem of hair loss. This effect on the number of hair cosmetics is marketed, both synthetic products and herbal products. Use of materials that are synthetic or herbal products has been widely produced. The use of synthetic substances in cosmetic products is less safe because it can cause side effects on long term use such as allergic effects, pathogenic, and carcinogenic (Yuan et al., 2016).

Several phytochemical and extract has been tested for the anticonvulsant (Ginting et al., 2018), antibacterial (Karsono et al., 2015; Masfria et al, 2016), antioxidant (Masfria et al., 2016; Nazliniwaty et al., 2016[a,b]; Nerdy and Manurung, 2018), antidiabetic (Nerdy, 2015[a]), antimalarial (Nerdy, 2015[b]; Nerdy 2017), anticancer (Nerdy, 2015[b], Nerdy et al., 2015; Nerdy et al., 2016), antihepatotoxicity (Tarigan et al., 2018), and antinephrolitiatic activity (Haro et al., 2017; Putra et al., 2018). Many medicinal plants have been used for the treatment of hair diseases from generation to generation (Begum et al., 2014). Herbs such as turmeric, and ginger are integral parts of ayurvedic formulations (Jain et al., 2016). However 6-gingerol(major component isolate from ginger), showed inhibition activity of hair growth (Miao et

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al., 2013). Many natural compounds are not efficacious as isolates but show activity in the combinations with other compounds in the extract. Figure 1 showed the difference between white ginger and red ginger.

Figure 1. Difference between white ginger and red ginger

People assume that red ginger has a better activity than white ginger. However, until now there is no scientific proof of hair growth activity of white ginger extract and red ginger extract. The purpose of this study is to conduct scientific tests on hair activity of white ginger extract and red ginger extract in the experimental animal.

2 MATERIALS AND METHODS

This research is an experimental research conducted in Pharmacology Laboratory, Faculty of Pharmacy, University of Sumatera Utara. Independent variable was ginger extract concentration and red ginger extract concentration. Dependent variable werehair growth time, hair length, and hair mass.

2.1 Tools and Materials

The tools used in this research were rats breeding equipment, calipers (Mitutoyo), analytical balance (Shimadzu),cutter (Kenko), scissors (Kenko), razor (Gillette), permanent marker (Snowman), beaker glass (Iwaki), erlenmeyer (Iwaki), measuring glass (Iwaki), stirring rod (Iwaki), petri dish (Iwaki), dropper pipette (Iwaki), spatula, aluminum foil (Klinpak), plastic wrap (Klinpak), blender (Philips), oven (Memmert), filter paper (Whatman), buchner funnel (Iwaki), and rotary evaporator (Buchi), waterbath (Wisebath), and camera (Samsung).

The materials used in this study were white ginger, red ginger, experiments animals were 27 male rats sprague dawley strain with aged 7 weeks to 8 weeks and weighed 150 g to 200 g, rats food (Best1), hexane (Merck), distilled water (Brataco), and tissue (Tessa), carbopol (Samirasachem), methyl paraben(Ueno), propyl paraben(Ueno), propylene glycol (Dow), minoxidil (Kirkland), triethanol amine (Merck).

2.2 Preparation of Extract

Extraction of white ginger and red ginger was modification from Nour et al (2017) extraction procedure.40 g fresh and grinded rhizomes was extracted by soxhletation method for 12 hours with n-hexane. The extract obtained were concentrated by rotary evaporator, dried by freeze dryer, stored in amber bottles, and kept at –4°C for further studies.

2.3 Experimental Design

This research was conducted by using completely randomized design with 9 treatments in male rats, each treatment was done 3 repetitions. The number of rats required for each treatment was determined using the Federer formula (n-1) (t-1) ≥15, where t denotes the number of treatments and n denotes the number of animals for each treatment (Pratisto, 2009). The treatment is NC: Without Treatment (normal control); (–)C: Blank Gel (negative control); (+)C: Minoxidil 2% Gel (positive control); WG1: 1% White Ginger Extract Gel; WG2: 2% White Ginger Extract Gel; WG3: 3% White Ginger Extract Gel; RG1: 1% Red Ginger Extract Gel; RG2: 2% Red Ginger Extract Gel; RG3: 3% Red Ginger Extract Gel.

2.4 Gel Formulation

Formulation of gel was modification from Gupta and Gupta (2017) formulation procedure. Eight different hair gel were prepared by a simple gel formulation preparation method based on carbopol gel. Gel formula for each treatment can be seen in Table 1.

Table 1. Gel formula for each treatment

Materials	–	+	Ginger			Red Ginger		
	NC	PC	G1	G2	G3	RG1	RG2	RG3
Minoxidil (g)	–	2.00	–	–	–	–	–	–
Ginger Extract (g)	–	–	1.00	2.00	3.00	–	–	–
Red Ginger Extract (g)	–	–	–	–	–	1.00	2.00	3.00
Carbopol (g)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Methyl Paraben (g)	0.50	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Propyl Paraben (g)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Propylene Glycol (mL)	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Triethanolamine (mL)	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Water (up to mL)	100	100	100	100	100	100	100	100

The carbopol was dispersed in 50 mL of distilled water. methyl paraben and propyl paraben were dissolved in 5 ml of hot distilled water, cooled, added propylene glycol, added extract (or minoxidil), mixed with carbopol solution, made up to 100 mL by distilled water with constant stirring, added triethanol amine and continued stirring until a transparent gel is formed.

2.5 Hair Growth Activity Test

Experiments animals were 54 male rats sprague dawley strain with aged 7 weeks to 8 weeksand weighed 150 g to 200 g. Mice were acclimatized for 2 weeks, fed with standard food and water, shaved hair on the back part (4 cm × 4 cm), and smeared with depilatory cream, made the middle box with permanent marker (2 cm × 2 cm, and kept for 24 hours. Each treatment with gel was smeared with 0.25 grams of gel at once daily in the morning (each application was rinsed using distilled water before smearing gel to avoid any previous gel still attached). The treatment was done for 21 days and observed hair growth time, hair length, and hair mass.

- Hair growth observations are performed daily with visual observations of two parameters: initial hair growth time (minimum time required to start hair growth in the skin area) and final hair growth time (minimum time required to cover the skin area with new hair).
- Hair length observation is done every week (7^{th} day, 14^{th} day, and 21^{st} day) by taking 10 hairs from each treatment box and measured in length using calipers.
- Hair mass observations were analyzed on the 21^{st} day by shaving the entire hair from each treatment box and weighed the mass using an analytical balance.

The data obtained is followed by one way analysis of variance with the least significant difference.

3 RESULTS AND DISCUSSIONS

Results obtained for extract yield were 1.2589% for white ginger and 1.3223% for red ginger. The extract obtained was further used for hair growth activity test compared to normal control, negative control, and positive control. Hair growth activity test for hair length and hair growth time for various treatment can be seen in table 2. Hair growth activity test for hair weight for various treatment can be seen in table 3.

Table 2. Hair growth activity test for hair length and hair growth time for various treatment

Treatment	Hair Length (mm) ± Standard Deviation (mm)			Initial Hair Growth Time	Final Hair Growth Time
	1st Week	2nd Week	3rd Week		
NC	1.7239 ± 0.0007^{A}	3.9552 ± 0.0016^{A}	6.5338 ± 0.0023^{A}	6^{th} Day	15^{th} Day
(–)C	1.7243 ± 0.0008^{A}	3.9606 ± 0.0015^{A}	6.5484 ± 0.0021^{A}	5^{th} Day	14^{th} Day
(+)C	1.7909 ± 0.0031^{B}	6.1863 ± 0.0039^{B}	10.1706 ± 0.0145^{B}	4^{th} Day	9^{th} Day
WG1	1.7456 ± 0.0021^{C}	4.5827 ± 0.0037^{C}	6.6306 ± 0.0136^{C}	6^{th} Day	12^{th} Day
WG2	1.7627 ± 0.0035^{D}	5.3778 ± 0.0043^{D}	7.8747 ± 0.0160^{D}	5^{th} Day	11^{th} Day
WG3	1.7883 ± 0.0049^{B}	6.1548 ± 0.0056^{B}	10.1096 ± 0.0199^{B}	4^{th} Day	10^{th} Day
RG1	1.7893 ± 0.0045^{B}	6.1712 ± 0.0057^{B}	10.1375 ± 0.0195^{B}	4^{th} Day	10^{th} Day
RG2	1.8164 ± 0.0054^{E}	6.8741 ± 0.0061^{E}	11.2641 ± 0.0214^{E}	3^{rd} Day	9^{th} Day
RG3	1.8315 ± 0.0059^{F}	7.4216 ± 0.0068^{F}	12.2641 ± 0.0237^{F}	2^{nd} Day	8^{th} Day

Table 3. Hair growth activity test for hair weight for various treatment

Treatment	Hair Weight (mg/mm^2) ± Standard Deviation (mg/mm^2)
NC	58.1523 ± 0.5263^{A}
(–)C	58.3886 ± 0.5332^{A}
(+)C	72.8623 ± 0.6716^{B}
WG1	62.5235 ± 0.5752^{C}
WG2	67.0735 ± 0.6013^{D}
WG3	72.4535 ± 0.6699^{B}
RG1	72.5652 ± 0.6703^{B}
RG2	76.6415 ± 0.6981^{E}
RG3	81.3182 ± 0.7136^{F}

White ginger extract concentration 1% and 2% have significant different weaker hair growth activity than minoxidil 2%; but on white ginger extract concentration 3% has not significant different (equal) hair growth activity to minoxidil 2%. Red ginger extract concentration 1% has not significant different (equal) hair growth activity to minoxidil 2%; but on white ginger extract concentration 2% and 3% have significant different weaker hair growth activity than minoxidil 2%. Red ginger extract has better hair growth activity than white ginger extract, this phenomenon might due to the hair growth activity phytochemical content (flavonoid) in red ginger extract was more than white ginger. Flavonoid play the main role on hair growth promoting through endothelial nitric oxide

synthase by inhibit nitric oxide production, or 5α-reductase (Rifkia et al., 2017).

Topical gel containing white ginger extract and red ginger extract with various concentration of extract showed the dose dependent hair growth activity. Higher concentration of extract in gel formulation has better hair growth activity, it showed by longer in hair length, faster in hair growth time and heavier in hair weight from 1st week, 2nd week and 3rd week. Herbal extract showed the dose dependent hair growth activity (Kanedi et al., 2017). Herbal was very effective for hair growth promotion without skin irritation. Hair growth activity of extract might be caused by the antioxidant effect and lead to improvement in blood flow to scalp (Semalty et al., 2010). Herbal might promote hair growth, enhance hair health, and effective for treating hair loss (Yu et al., 2017). Herbal extract treatment could proliferated human dermal papilla cells, significantly stimulated the expression of Ki-67 protein and the messenger ribonucleic acid levels of hepatocyte growth factor (Boisvert et al., 2017). Herbal might be an alternative for synthetic drugs used for alopecia without any side effect.

4 CONCLUSIONS

White ginger extract has weakerhair growth activity than minoxidil, but red ginger extract has stronger hair growth activity than minoxidil. Evaluation of hair growth activity with hair growth time, hair length, and hair mass parameter showed significant difference between white ginger extract and red ginger extract. Red ginger extract showed better hair growth activity than white ginger extract.

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