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Potential of Hibiscus Rosa-Sinensis L. and *Baccaurea Racemosa* Extract as a Hair Growth with Tail Suspension Test

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ABSTRACT

Alopecia is the loss of excess hair that causes temporary or permanent baldness. Physical and emotional stress are the most common causes. This study aims to determine the potential of Hibiscus rosa-sinensis L. and Baccaurea racemosa extracts on stress-induced hair growth in alopecia. Stress induction in male Wistar albino rats was obtained using the tail suspension test (TST) method. The results of the chemical content test were the total phenol content test (4,223.06 mg/100 g), total flavonoid (10,383.12 mg/100 g) and DPPH free radical reduction of B. racemosa (EB) extracts (1,593.17 mg/L GAEAC) were greater than those of the H. rosa-sinensis L. (EH) extract. The longest hair and highest hair density were obtained in the Minoxidil 2% group. While the most hair follicles were found in the group given the combination of EH and EB = 1: 1. The content of phenolic and flavonoid substances affects the hair growth activity..

Keywords *: Hair growth, Hibiscus rosa-sinensis, Baccaurea racemosa.*

INTRODUCTION

Hair is one of the aesthetic aspects of a person. Usually, each person will experience hair loss of 70–100 strands per day (Dawid-Pać, Urbańska, Dębosz, & Nowak, 2014). The severity of hair loss can be divided into three categories, namely: mild if you lose less than 40 hairs per day; moderate if you lose 40-100 strands per day; and more than 100 strands per day is categorized as severe. Alopecia is excessive hair loss that causes baldness. This condition can affect a person's psychology. Alopecia affects 66% of men and 40% of women over 35 years old (Semwal, et al., 2015). Alopecia can be temporary or permanent. Various factors can influence hair loss that triggers alopecia, including hormones, systemic diseases, drugs, genetic tendencies, local skin disorders, post-acute illness, nutritional deficiencies, and stress (Amin & Sachdeva, 2013). However, the most common causes are physical and emotional stress, thyroid, or other hormonal imbalances (Semwal, et al., 2015).

Stress is a physical and psychological response to risks, challenges, or impediments that can impair metabolism and other bodily functions. The production of free radicals is also influenced by stress. The antioxidant defense system is the body's strategy for protecting cells from the damaging effects of free radicals. Enzymatic and non-enzymatic

defensive systems are involved in this system. Antioxidants act in the oxidation of intracellular and extracellular biomolecules by delaying or inhibiting them. Antioxidants aid in the battle against free radical-induced oxidative damage. Disturbances and cell damage might develop when the body's defense mechanisms cannot resist the excess of free radicals (Kawamura & Muraoka, 2018).

Stress does not cause hair loss directly; behavioral and mental changes trigger it. Neurogenic inflammation develops in neurons during stressful situations, inhibiting the effects of nerve growth factor (NGF), which is involved in hair follicle development. According to research on stress induction in experimental mice, the release of NGF by sensory nerves and immune cells in hair cells stimulates mast cells. In addition, it triggers macrophage migration to surrounding hair follicles. As a result, hair loss occurs due to increased hair follicle apoptosis and hair follicle stem cells (Likhitkar, Shakur, Bansal, & Pande, 2018).

Various preparations can be used to treat alopecia, one of which is Minoxidil 2%. However, the use of synthetic compounds causes side effects in the long term. Herbal-based treatment has advantages in terms of side effects and costs and increases the convenience of its use in alopecia patients (Likhitkar, Shakur, Bansal, & Pande, 2018). Therefore, finding natural compounds that can increase hair growth without other adverse effects is necessary.

The hibiscus plant (*H. rosa-sinensis* L.) has long been used to promote hair development and prevent premature greying, hair loss, and scalp conditions (Al-Sanafi, 2018; Singh, Tailang, & Pathak, 2017). Although *B. racemosa* has high antioxidant activity (Wulansari & Chairul, 2011), its effect on hair growth is not yet understood. Antioxidants play a role in countering the damaging effects of free radicals caused by stress. This study aimed to ascertain the effect of the two extracts alone and combined on hair growth under stress. The findings of this study can offer rational support for using natural substances in the treatment of stress-induced alopecia.

MATERIAL AND METHODS

Materials and Instrument

H. rosa-sinensis L. was obtained from Bokashi Farm (Denpasar), and B. *racemosa* was obtained from the Karangasem area. The two research materials have been identified at the Bali-LIPI Botanical Garden Plant Conservation Center "Eka Karya" (B-153/IPH.7/AP/IV/2020). The reagents used include 96% ethanol, Folin-Ciocalteau's, $CaCO_3$, gallic acid (E. Merck), $AlCl_3$, methanol, acetate buffer, quercetin, and DPPH.

Thirty rats of Wistar or known as laboratory rats, weighing 150-200 grams, were used in this study. The acclimatization process lasts for seven days. Experimental animals were

given standardized concentrate feed and drinking water ad libitum.

Extraction

H. rosa-sinensis (EH) and *B. racemosa* (EB) leaves were cleaned and then dried. The leaves were extracted by maceration with ethanol 96% (1:10 w/v) for 2 x 24 hours with constant stirring and evaporated by a rotary evaporator.

Total phytochemical content

Total phenol and flavonoid content was measured using Widodo's adapted method with slight modifications (Widodo, Sismindari, Asmara, & Rohman, 2019). A 40 µl of plant extract (1 mg/ml; 1 mg dissolved in 1 ml methanol) was mixed with 360 µl of distilled water and 100 µl of Folin-Ciocalteau's, and the solution was shaken and left for 2 minutes. The reaction was neutralized using 500 µl of 10% CaCO3 and mixed until homogeneous. The mixture was incubated for 20 minutes at 40°C. A 150-µl test solution was included in the microplate, and the absorbance was measured at wavelength 732 nm. The total phenol content were expressed as mg gallic acid equivalent/g of the extract through linear regression prepared from gallic acid standards at various concentrations (0, 5, 10, 15, 20, and 25 µg/ml).

The total flavonoid content was measured by a mixture consisting of 100 µl extract, 150 µl solution of 0.1 M $AlCl_3$ (blank without $AlCl_3$ and replaced with methanol 150 µl), 350 µl of aquadest, 250 µl acetate buffer (pH 3.8), and added with methanol up to a total volume of 1,250 µl. The test solution was incubated at 35°C for 30 minutes, and the absorbance was measured with a UV-Vis spectrophotometer at 435 nm. Total flavonoid content were expressed as quercetin equivalents per g extract via generating a standard curve with a series of concentrations 0–100 µg/ml of quercetin.

DPPH Radical Scavenging Activity

Kikuzaki et al. (2002) method was slightly modified based on DPPH radical-scavenging activity ((Kikuzaki, Hisamoto, Hirose, & Akiyama, 2002). The solution of DPPH 0.4 mM was prepared by dissolving 15.8 mg of DPPH with 100 mL of methanol p.a, and then 1 mL of this solution was mixed with 4 mL of extract, fractions, and quercetin (standard) at different concentrations. A control was prepared by adding 1 mL of DPPH 0.4 mM with 4 mL of methanol p.a. They were shaken and stood at room temperature for 30 mins. The absorbance of the solutions was measured at 515 - 516 nm with methanol as a blank using a UV-Visible spectrometer (Biochrome SN 133467). The DPPH free radical scavenging activity of the sample was calculated using the following equation

Hair Growth Activity Test

Before treatment, rats were induced by stress using the Tail Suspension Test (TST) method for five days. TST testing was adopted from Can's method. In short, TST was

performed by hanging the rat's tail using tape on a box measuring 15 cm in length, 11.5 cm in width, and 55 cm in height for 6 minutes every day. The length of the rat's nose from the floor was 20-25 cm. On the sixth day after stress induction, the mice's fur was shaved to a size of 2.5 x 2.5 cm

Each treatment group received 1 mL of the extract, which they applied to the denuded area for 21 days. Skin specimens were taken for follicular examination on days 7, 14, and 21 of therapy. Hair growth activity was measured using hair length, density, and histology assays. The hair length of experimental animals was determined by randomly extracting hair from the place where the hair was removed with sterile tweezers. On day 21, hair density was determined by shaving 1 cm^2 of hair and manually counting the number of strands obtained. A skin biopsy was used to do a histological examination.

RESULTS AND DISCUSSION

In this study, only the total phenol content, total flavonoid content, and free radical scavenger DPPH from EH and EB were carried out as a phytochemical analysis to determine their antioxidant activity.

B. racemosa has been shown to have high antioxidant activity (91.23% ± 0.02) (Wulansari and Chairul, 2011). In this study, EB (1,593.17 mg/L GAEAC) was found to have a higher ability to suppress free radicals than EH (Table 1). Compared to EH, EB has more phenol and flavonoid components. The concentration of flavonoids and polyphenolic components in B. *racemosa* influences its antioxidant activity (Gunawan, Chikmawati, Sobir, & Sulistijorini, 2016; Permatasari, Riyanto, & Rohman, 2019; Widodo, Sismindari, Asmara, & Rohman, 2019). B. *racemosa* has compounds that can decrease free radicals and inhibit lipid peroxidation (Permatasari, Riyanto, & Rohman, 2019). Because they may deactivate alkyl peroxyl radicals and superoxide molecules, flavonoids are known to operate as free radical scavengers. Polyphenols can also operate as reducing agents, hydrogen donors, oxygen radical scavengers, and metal chelators. The hydroxyl group in phenolic compounds plays a key role in their antioxidant action (Wulansari & Chairul, 2011).

Staining with hematoxylin and eosin was applied to a cross-section of the skin of each treatment group. The hair follicles in each treatment group are depicted in Figure 1. An outer root sheath (ORS) and an inner root sheath make up hair follicles (IRS). After 21 days of treatment in all treatment groups, it is known that the average length of hair obtained in the Minoxidil 2% group is 13.4 mm. The extract-treated groups (EH, EB, and EHB) showed that the average hair length in the EH group was 12.94 mm. Based on statistical analysis using one-way anova, it was found that there was no significant difference

Table 1. Phytochemical Test of Extract of H. rosa-sinensis L. and Extract of B. *racemosa*.

Extract	Total Phenol (mg/100 g)	Total Flavonoids (mg/100 g)	Suppression of DPPH Free Radicals (mg/L GAEAC)
EH	1,984.30	9,202.82	673.65
EB	4,223.06	10,383.12	1,593.17

Table 2. Hair Growth

Group	Hair Growth (mm) ± S.D.		
	Day 7	Day 14	Day 21
Control	1.87 ± 0.06*	2.81± 0.09*	9.26 ± 1.14*
Minoxidil 2%	6.14 ± 0.08*	12.23 ± 0.68*	13.4 ± 0.19*
EH	2.78 ± 0.02	4.83 ± 0.39*	12.94 ± 0.39
EB	2.46 ± 0.04*	5.73 ± 0.17*	7.75 ± 0.23*
EHB	2.75 ± 0.05	3.2 ± 0.09	12.58 ± 1.08

*Shows a significant difference ($p < 0.05$) in the same column

Table 3. Hair Density

Group	Hair Density (strand/cm^2) ± S.D.
Control	800 ± 61.10
Minoxidil 2%	1200 ± 35.12
EH	1100 ± 32.02
EB	950 ± 56.86
EHB	1000 ± 30.55

Table 4. Number of Hair Follicles

Group	Hair Follicles ± S.D.		
	Day 7	Day 14	Day 21
Control	19 ± 2	40 ± 4.58*	31 ± 4.36
Minoxidil 2%	58 ± 4.36	52.67 ± 9.29*	25.33 ± 3.06*
EH	19 ± 6.56	32.67 ± 4.73	10 ± 3.61
EB	16 ± 3.46	33.33 ± 2.52*	6.33 ± 0.58*
EHB	26 ± 7.55	55.33 ± 16.07	41.33 ± 2.89*

*Shows a significant difference ($p < 0.05$) in the same column

between the EH and EHB groups ($p > 0.05$). EH is known to increase blood flow to the scalp and trigger hair growth (Semalty et al., 2010). Increased blood circulation and widening of blood vessels can increase nutrient intake, affecting hair growth (Febriani, Elya, & Jufri, 2016). The activity of EH on hair is supported by its flavonoid content. Flavonoids can increase hair growth by strengthening capillary walls in hair follicle blood vessels (Upadhyay, Upadhyay, Vinode, & Dixit, 2013).

Figure 1. Hair follicles on a cross section of the skin with haematoxilin eosin staining, 1 view with a magnification of 10 x 10.

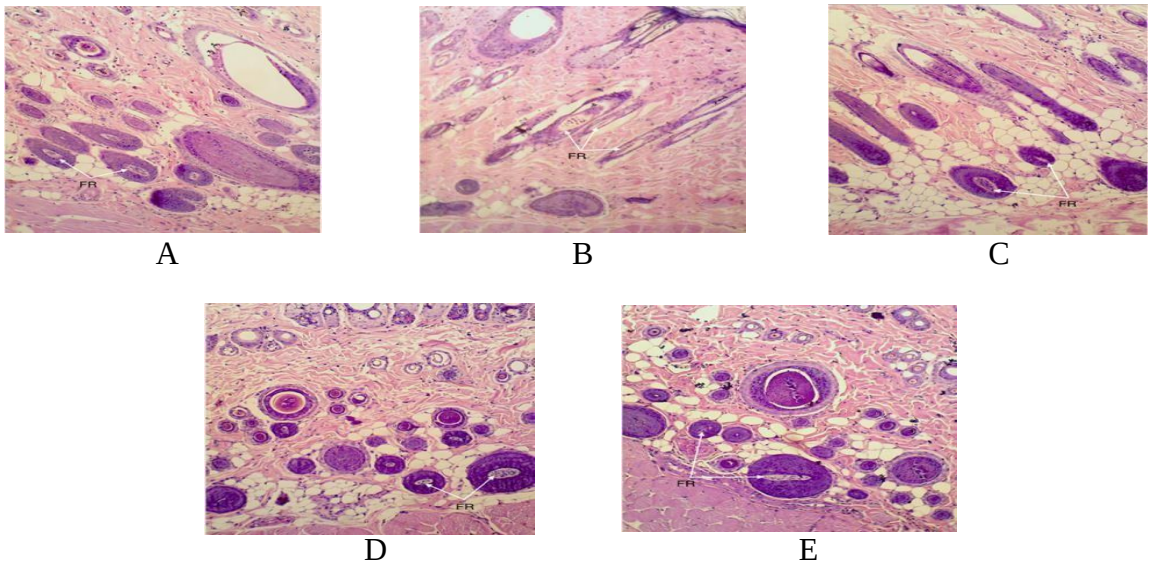

Figure 1. Hair follicles on a cross section of the skin with haematoxilin eosin staining, 1 view with a magnification of 10 x 10.

Note: (A) cross-section of the skin of control mice, (B) cross-section of rat skin with 2% minoxidil, (C) rat skin cross section treated with EH, (D) cross section of rat skin with EB, (E) cross section of rat skin with a combination of EH and EB (1:1), (FR) the follicle root of hair

Table 3 presents the number of hair follicles obtained from histology tests. After 21 days of treatment, it was found that most hair follicles were found in rats given the combination of EH and EB. Hair growth in the minoxidil group was the fastest compared to other groups. The combination of EH and EB showed significant results after 21 days of observation. It is made possible by the synergistic effect of the antioxidant activity of the two extracts, which stimulates hair growth.

CONCLUSION

The *H. rosa-sinensis* extract (EH) is known to contain phenolic compounds (1,984.30 mg/100 g) and flavonoids (9,202.82 mg/100 g) and has DPPH-reducing activity (673.65 mg/L GAEAC). Meanwhile, *B. racemosa* extract (EB) exhibits stronger DPPH lowering activity (1,593.17 mg/L GAEAC) than *H. rosa-sinensis* extract and is reported to contain phenolic compounds (4,223.06 mg/100 g) and flavonoids (10,383.12 mg/100 g). This study indicated that *H. rosa-sinensis* L. and *B. racemosa* extracts contain phenolic and flavonoid compounds and exhibit antioxidant activities. The longest and densest hair were obtained in the Minoxidil 2% group. In contrast, most hair follicles were found in the groups given the EH and EB. The antioxidant activity of EH and EB has a synergistic impact, which influences their activity on hair growth. However, further studies are necessary to determine the chemical composition of *H. rosa-sinensis* L. and *B. racemosa* extracts responsible for the hair growth activities.

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