

Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
 Open Access Macedonian Journal of Medical Sciences. 2020 Feb 15; 8(A):89-92.
<https://doi.org/10.3889/oamjms.2020.4211>
 eISSN: 1857-9655
Category: A – Basic Sciences
Section: Pharmacology



The Potency of *Hibiscus rosa-sinensis* Linn. Leaves Ethanol Extract as Hair Growth

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Abstract

Edited by: Igor Spiroski
Citation: Putra IB, Jusuf NK, Sumantri IB. The Potency of *Hibiscus rosa-sinensis* Linn. Leaves Ethanol Extract as Hair Growth. Open Access Maced J Med Sci. 2020 Feb 15; 8(A):89-92.
<https://doi.org/10.3889/oamjms.2020.4211>
Keywords: *Hibiscus rosa-sinensis*; Hair growth; Leaves; Extract
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Received: 17-Dec-2019
Revised: 07-Feb-2020
Accepted: 13-Feb-2020
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Funding: USU Grant (No. 2590/UN5.1.R/PPM/2018), Research Institute, TALENTA, Universitas Sumatera Utara
Competing Interests: The authors have declared that no competing interests exist
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INTRODUCTION: Hair is one of the organs and crowns for all people. Hair loss will really bother for most people. One of the plants that can be used as hair growth is *Hibiscus rosa-sinensis* Linn.

OBJECTIVE: The objective of the study was to find the potency of *H. rosa-sinensis* leaves ethanol extracts as hair growth.

METHODS: In this research, simplicia and extracts were made from hibiscus leaves using ethanol as a solvent. Simplicia and extracts were tested for phytochemical screening and characterization of each. The extract obtained was tested *in vivo* with animals test by observing mouse's hair growth for 25 days of observation.

RESULTS: Simplicia and ethanol extract of *Hibiscus* leaves contain flavonoids, alkaloids, glycosides, tannins, and steroids/terpenoids. The characteristics of *Hibiscus* leaves simplicia and extract had water content (9.71% vs. 10.65%), water-soluble extract content (19.91% vs. 27.58%), ethanol-soluble extract content (37.86% vs. 49.51%), total ash content (7.96% vs. 9.46%), and acid-insoluble ash content (0.78% vs. 1.28%). *In vivo* testing with animals, ethanol extract of *Hibiscus* leaves had an effect as a hair growth until the 25th day with an average concentration of 2.5% (14 mm), 5% (16 mm), and 10% (19 mm).

DISCUSSION: The extract of *Hibiscus* leaves that showed the presence of carbohydrate, alkaloid, flavonoid, steroids, protein, tannin, and amino acids. The presence of active constituents such as flavonoids and tannins may be responsible for hair growth activity.

CONCLUSION: The ethanol extracts of *H. rosa-sinensis* leaves can increase hair growth, at optimum concentrations of 10% extract.

Introduction

Hair is one of the organs and crowns for everyone [1]. One of the hair problems is hair loss. Hair loss can reduce a person's appearance and its main function as protection of the body and head from the environment. Hair loss is not a life threatening, but can directly affect self-confidence which can subsequently be psychological. Hair loss causes baldness and becomes a quite worrying problem [2], [3]. Normally, the average person loses 50–100 strands of hair everyday. However, if the hair loss is more than 100 strands/day and occurs continuously, then this is a characteristic of unhealthy hair [4], [5].

Nearly 85% of men will experience hair thinning at the age of 50 years and some men experience hair loss before the age of 21 years [6]. In the United States, hair loss affects nearly 50 million people and about 20 million of them are women. The etiologies are vary, classified as endogenous due to systemic, hormonal, nutritional status, intoxication, and genetic disorders, and exogenous due to stimuli from the environment,

as well as hair cosmetics [7], [8]. Stimuli from environment and hair cosmetics are rarely recognized for their impact on hair health. Environmental factors can include exposure to heat, sunlight, pressure, X-ray radiation, and water on the hair, while hair cosmetics refer to hair care and styling such as shampoo. Hair loss due to these two factors can occur through the mechanism of hair shaft fracture, loss, and baldness [3], [8], [9].

Treatments of hair loss depend on the type or damage level of hair loss that occurs. Some conditions cannot be treated at all, but some can be treated and grow back if the etiology is removed [10]. Hair loss can be prevented by treatment from outside or inside. Treatment from the outside can be done using topical preparations in the form of ointments or hair care solutions while treatment from within can be done by consuming drugs and injections to stop hair loss and accelerate hair growth [5], [11]. Various types of therapies that can be used to treat hair loss are corticosteroids, immunotherapy, phototherapy using ultraviolet B, minoxidil, and cyclosporine [11]. The use of herbs as a hair growth shampoo and treatments of hair

loss is growing. One of the plants that can be used as a hair growth is *Hibiscus* (*Hibiscus rosa-sinensis* Linn.).

Petroleum ether extract from *H. rosa-sinensis* leaves and flowers has a potential as a hair regrower both *in vivo* and *in vitro*. The concentration of 1% extract that given topically affected the growth of white hair rat during 30 days observation [12]. Another study by Upadhyay *et al.* reported a hair growth effect on the ethanol extract of the *H. rosa-sinensis* flowers [13]. Based on the description above, we conducted an *in vivo* research on the efficacy of *Hibiscus* leaves ethanol extract for increasing hair growth.

Objective

The objective of the study was to find the potency of *H. rosa-sinensis* leaves ethanol extracts as hair growth.

Materials and Methods

Preparation of *simplicia* and extract

Hibiscus leaves (*H. rosa-sinensis* Linn.) originated from Deli Serdang, North Sumatra. The leaves are washed clean, dried, and mashed to obtain *simplicia* powders. *Simplicia* powders were extracted in 96% ethanol by percolation. The extract is evaporated until a thick extract was obtained then freeze dryer. Dry extracts are packaged in dark-colored bottles and stored in a refrigerator at 4°C.

Phytochemical Screening and Physicochemical Analysis

Simplicia and extract were subjected to phytochemical screening by identification using different spraying reagent for particular compounds, such as dragendorff for alkaloids, AlCl_3 for flavonoid, FeCl_3 for tannin, Lieberman Burchard for steroid and sulfuric acid for saponin/triterpenoid. Beside that, the *simplicia* and extract were also analyzed for physicochemical properties, such as water and ethanol-soluble extract, water content, total ash content and acid-insoluble ash content.

In vivo test

Animals that are used in this test were white Wistar strain rats with weighing 150–200 g. Test animals are acclimated overnight for 1 week with standard food and water and 12 h of bright lighting and 12 h of dark at temperature ($25 \pm 3^\circ\text{C}$). All research protocols have been approved by the research ethics committee. Rat that used for the test is applied with depilatory cream to clean its hair that has begun to grow and is left for 24 h.

After that, about 0.25 g of the test material in cream is applied twice a day (morning and evening) for 25 days in the test area. The average hair length is obtained by measuring 10 strands of hair on the 5th, 10th, 15th, 20th, and 25th days after applying. Some hair is pulled randomly by the roots and straightened first with a glass object and selected 10 whole hairs that are contained follicles and tapered ends. Results are expressed as the average hair length \pm SD of 10 hairs. Hair length and thickness were observed under a light binocular microscope with a magnification of the ocular lens 10 times and objective lens 4 times so that a conversion factor of 25.2 μm was used. Hair thickness is measured by pulling 10 strands of hair and measuring the width at three different places and the average value is taken.

Ethical approval

The protocol of this study was approved by the Health Research Ethical Committee, University of Sumatra Utara, H. Adam Malik General Hospital, Medan, Indonesia.

Results

The results from the process of drying *Hibiscus* leaves (*H. rosa-sinensis*) into *simplicia* obtained 13.5% materials. Extraction with 96% ethanol solvent using percolation method was obtained 12.6% extract materials.

Characteristics of *simplicia* and extract of *Hibiscus* leaves (*H. rosa-sinensis*)

The characteristics of *Hibiscus* leaves *simplicia* had 9.71% moisture content, 19.91% water-soluble content, 37.86% ethanol-soluble content, 7.96% total ash content, and 0.78% acid-insoluble ash content, whereas the characteristics of *Hibiscus* leaves extract have 10.65% moisture content, 49.51% ethanol-soluble content, 27.58% water-soluble content, 9.46% total ash content, and 1.28% acid-insoluble ash content (Table 1).

Table 1: The characteristics of *Hibiscus* leaves *simplicia* and extract

Tests	<i>Simplicia</i> (%)	Extract (%)
Moisture content	9.71	10.65
Ethanol-soluble content	37.86	49.51
Water-soluble content	19.91	27.58
Total ash content	7.96	9.46%
Acid-insoluble ash content	0.78	1.28

Phytochemical screening of *Hibiscus* leaves (*H. rosa-sinensis*) *simplicia* and extract

The results of phytochemical screening for both *simplicia* and extract of *Hibiscus* leaves were contained alkaloids, tannins, saponins, triterpenes/steroids, flavonoids, and glycosides (Table 2).

Table 2: Phytochemical screening of *Hibiscus leaves simplicia* and extract

Phytochemical screening	Simplicia	Extract
Alkaloids	+	+
Tannins	+	+
Saponins	+	+
Triterpenes/steroids	+	+
Flavonoids	+	+
Glycosides	+	+

In vivo test

The *in vivo* test was carried out on white rats for 25 days as shown in Figures 1 and 2. The results of *in vivo* test showed that mean hair length after 25 days application of 2.5%, 5%, and 10% *Hibiscus* leaves ethanol extract on white rats were 14 mm, 16 mm, and 19 mm, respectively (Table 3 and Figures 1 and 2).

Table 3: The results of hair growth of *in vivo* test

Day	Ethanol extract of <i>Hibiscus</i> leaves				
	Normal (mm)	Base (mm)	2.5% (mm)	5% (mm)	10% (mm)
D-0	0	0	0	0	0
D-5	2	3	3	3	1
D-10	3	4	5	5	5
D-15	6	10	10	12	9
D-20	10	11	12	14	16
D-25	12	13	14	16	19

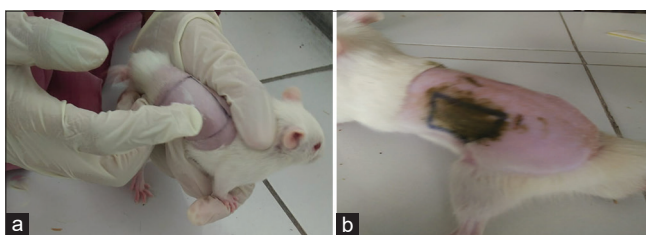


Figure 1: Application of (a) base cream and (b) ethanol extract of *Hibiscus* leaves

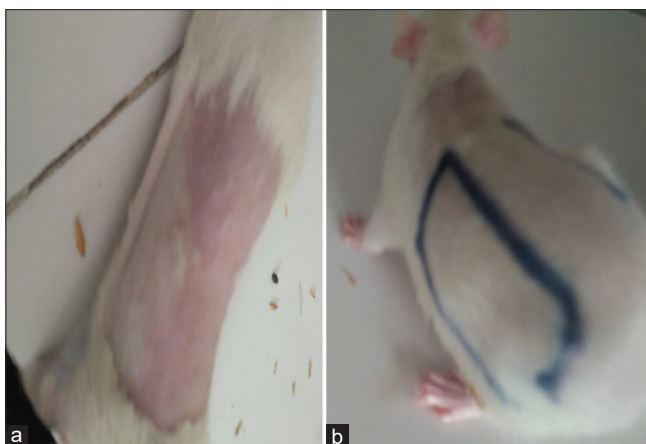


Figure 2: (a) D-0 and (b) D-25 post-application of 5% *Hibiscus* leaves ethanol extract

flavonoids, and glycosides. This results supported by the phytochemical study by Singh *et al.* on the extract of *Hibiscus* leaves that showed the presence of carbohydrate, alkaloid, flavonoid, steroids, protein, tannin, and amino acids [14].

The effect of the ethanol extract of *Hibiscus rosa-sinensis* leaves was investigated in stimulating hair growth in shaved skin white Wistar strain rats. The *in vivo* test results showed that mean hair length after 25 days application of 2.5%, 5%, and 10% *Hibiscus* leaves ethanol extract was 14 mm, 16 mm, and 19 mm, respectively, which are better than placebo and control groups. For the placebo and control groups, respective mean hair growth was 12 mm and 13 mm. A study by Adhirajan *et al.* reported the effect of *Hibiscus rosa-sinensis* leaves and flowers extract after being applied locally for 30 days on shaved skin of albino rats, in the form of 1% extracts in liquid paraffin. The results of extract from leaves showed greater influence on hair length than extract from flowers. After 30 days, 17 mm of hair growth in animals subjected to the activity of leaf extract, compared to 15.8 mm of hair growth in the group subjected to the activity of flower extract was obtained. For the placebo and control groups, respective values were 14.5 mm and 13.6 mm. At the end of the study, it was observed that 67% of all hair follicles stimulated by that extract were in the anagen phase [12].

Another study by Rahmawati *et al.* reported activity test on the single extract or combination of *Hibiscus* leaves and biji saga (*Abrus precatorius* L.) hair tonic showed the length and the thickening of hair. In 1%, single extract was effective for hair growth and in combination extract, 0.5% of each other extract was also effective for hair growth [15]. A study by Singh *et al.* also reported the hair growth activity of *H. rosa-sinensis* and *Calotropis gigantea* leaves on stress-induced alopecia rats. They reported that mean hair length in hydroalcoholic extract of *Hibiscus* group was 5.35 ± 0.13 mm, while in hydroalcoholic extract of *C. gigantea* group (5.460 ± 0.09 mm) and in polyherbal hydroalcoholic extract of *H. rosa-sinensis* and *C. gigantea* groups (5.820 ± 0.06 mm). Moreover, mean hair density in hydroalcoholic extract of *Hibiscus* group was $12,230 \pm 258.84/\text{cm}^2$, while in hydroalcoholic extract of *C. gigantea* group ($12,900 \pm 223.60/\text{cm}^2$) and in polyherbal hydroalcoholic extract of *H. rosa-sinensis* and *C. gigantea* groups ($12,360 \pm 288.09/\text{cm}^2$). The presence of active constituents such as flavonoids and tannins may be responsible for hair growth activity [14].

Discussion

Phytochemical screening results from the extract of *Hibiscus* leaves (*H. rosa-sinensis*) contained alkaloids, tannins, saponins, triterpenes/steroids,

Conclusion

Results of this study proved that the ethanol extracts of *H. rosa-sinensis* leaves can increase hair

growth, optimum concentration at concentrations of 10% extract.

Acknowledgment

We would like to thank the Vice Rector III of Research, Community Service and Cooperation, Universitas Sumatera Utara and Head of Research Institute, Universitas Sumatera Utara for their support.

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