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HAIR GROWTH PROMOTING ACTIVITY OF LANGIR BARK (*Albizia saponaria* LOUR.) ETHANOL EXTRACT: *In-vivo*ASSAY

Himaniarwati[1,2,✉], Muhammad Arba[4], Yasmiwar Susilawati[3] and Resmi Mustarichie[1]

[1]Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Universitas Padjadjaran, Indonesia
[2]Departement of Pharmacy, Universitas Mandala Waluya, South-East Sulawesi, Indonesia
[3] Department of Pharmacy Biology, Faculty of Pharmacy, Universitas Padjadjaran Indonesia
[4]Faculty of Pharmacy, Halu Oleo University, Kendari, Indonesia
✉Corresponding Author: himaniarwatie@gmail.com

ABSTRACT

Hair loss and baldness are problems that are often found in clinical dermatology terms. This study aims to determine the ethanol extract of *Albizia saponaria* stem bark as a hair growth stimulant. The barks of *Albazia saponaria* has collected from the Tolaki tribe, South Konawe, Southeast Sulawesi. Extraction was carried out by maceration and it's fractionated by using liquid-liquid extraction methods. Phytochemical screening according to the Farnsworth method. The hair growth activity for male rabbits is using the modified Tanaka method. The phytochemical screening showed that the ethanol extract contained tannins, alkaloids, flavonoids, saponins, phenols, and triterpenoids. The results of the hair growth activity showed that the ethanol and water extract of concentrations of 5%, 10%, 15%, and 20% significantly fertilized the rabbit male hairs for 24 days. The ethanol extract and water fraction with a concentration of 15% albizia barks have been showing a p-value <0.05. The results showed that the ethanol extract and water fraction of *Albizia saponaria* barks were effective for rabbit hair growth and *Albizia saponaria* has the potential for hair growth.

Keywords: Hair Growth, Ethanol extract, *Albizia sponaria*, Phytochemical screening.

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INTRODUCTION

Hair loss and baldness are diseases that are often found in terms of clinical dermatology. There are a number of products of drugs that are claimed to increase hair growth such as using synthetic compounds or natural products. The natural products for hair growth have been reported in several studies such as the development and evaluation of VCO-based herbal hair tonic[1], Hair growth promoting activity of Eclipta alba[2], and Anti-Alopecia Activity of Dadap (Erythrina variegata L.) Leaves Ethanol Extract[3] and evaluation of hair growth-supporting activity of three polyherbal formulations using the extract of Cicer arietinum Linn., Ocimum sanctum Linn. and Cyperus rotundus Linn.[4] This research gives great results of the alternative curing for baldness treatments. However, the assessment of the products, including a spacious qualitative and quantitative approach, depicting hair growth, was not satisfying.[5] Some of the synthetic drugs available for hair growth include minoxidil, finasteride, and spironolactone.[6] These drugs have been clinically tested to grow hair but were provided systemic and locally irreversible.[7] Currently, many products made from natural ingredients have been developed due to fewer side effects and better formulation methods.[8] Natural ingredients that are thought to grow hair is the genus Albizia. Albizia compounds from several species are saponins, triterpenoids, flavonoids, alkaloids, phenolic glycosides, and others. Previous research on the Albizia lebbeck plant showed the presence of secondary metabolites, namely tri-O-glycosides kaempferol flavonoids, and quercetin identified from the leaves of Albizia lebbeck.[9] Flavonoids from some plants have an activity that increases hair growth by monitoring the capillary walls of the tiny blood vessels that supply hair follicles.[10] Albizia Saponaria is an Indonesian medicinal plant mainly used by the Tolaki tribe in Southeast Sulawesi Indonesia. The use of natural ingredients is based on habits passed down from generation to generation as an alternative treatment and prevention of head diseases. However, Albizia Saponaria Lour in scientific data of the existence of

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secondary metabolites that have hair growth activity has been proved yet. The study of the chemical compounds and activity of the ethanol extract of *Albazia saponaria* barks as stimulant hair growth to seek alternative repairing against baldness.

EXPERIMENTAL

Extraction Method

The langir bark was dried out direct from sunlight and blended into a coarse powder. The 96% ethanol extract was obtained by maceration for 3x24 hours and filtered using filter paper. Remaceration was carried out again 2 times. The solvent that had been collected was concentrated with a rotary evaporator at 45° C and then dried using a hair dryer. Next process, the macerate was concentrated using a rotary evaporator at low pressure and temperature preventing the damage of secondary metabolite compounds due to overheating. The ethanol extract obtained was then partitioned by liquid-liquid partition using water, n-hexane, and ethyl acetate solvents.

Phytochemical Screening

Phytochemical screening tests were performed to examine the presence of alkaloids, tannins, polyphenols, flavonoids, sesquiterpenoids, monoterpenoids, steroids, triterpenoids, quinones, saponins in simplicia which provide hair growth activities based on the Farnsworth method.

Alkaloid Test

2 ml of the extract was mixed with 2N hydrochloric acid and shaken. Part of the acid layer obtained was then divided into 3 parts. The first part was used as a blank, the second part was reacted with Dragendroff's reagent and observed the formation of orange, and the third part was used to be reacted with the Mayer reagent and the presence of white deposits observed

Flavonoid Test: A few milliliters of 5N hydrochloric acid mixed with Mg metal were added to the sample and then heated. The appearance of red color that could be drawn by amyl alcohol indicates the presence of flavonoids.

Saponin Test

The sample was heated with distilled water in a test tube and filtered. The filtrate was shaken vigorously to the formation of stable persistent froth. The formation of foam with a height of at least 1 cm and persistent for several minutes and was not lost when 1 drop of hydrochloric acid was added showed the presence of saponins.

Tannins Test

In the test tube containing the sample added distilled water and then heated and filtered in hot conditions. 1% gelatin solution was added to the filtrate. The appearance of white and sediment indicated the presence of tannins in the test samples.

Steroid and Triterpene Test

The sample was extracted with ether, then evaporated using a vaporizer plate to dry. Added Lieberman Burchard reagent on the residue. The presence of the triterpenoid group indicated that in purple color, whereas the presence of the steroid group compounds formed a blue-green color.

Quinone Test

Screening for Quinone 1ml of the extract was added with 1ml of concentrated H2SO4. The appearance of the red color shows the presence of Quinone.

Polyphenol Test

The extract was dissolved in 5 ml of distilled water, then the tube was heated and filtered in hot conditions. The obtained filtrate was added to an iron(III) chloride solution. The presence of polyphenol compounds was shown by the black and blue color.

Experimental Animals

The experimental animal used was a white male Angora strain, 4-5 months old, obtained from the Faculty of Animal Husbandry, University of Halu Oleo Kendari. The ethics license for animal research applied to

the Health Research Ethics Committee Research and development department at Halu oleo University. In preparation of experimental animals, three male Rabbits were used, calculated using Federer rule[11], non-anatomical defects, and nonillness, 4-5 months old. Before use, rabbits were acclimatized for 7 days in order to adapt themselves to the atmosphere and new treatment, then rested for 24 hours.

Dosage Preparation

Preparation of langir bark extract concentrations of 5%, 10%, 15%, and 20%. NaCMC 500 mg in a mortar added in hot water, left until soluble, stirring vigorously that completely blended and added the extract, mixing so that homogeneous and then added aqua dest to 100 mL.

Hair Growth *In vivo* Determination

The hair growth test is used based on a method of Tanaka[12] which had been used and modified by others.[2,13] In this research experimental animal used is a rabbit. The back of the hair rabbit was sheared to clean then divided into 7 boxes with size 2x2 cm and given the following treatment. The first stage of the test was too identified for the effective dose in langir bark extract. The rabbit's back was drawn into 7 boxes and each box was smeared with Langir bark extract of 5%, 10%, s15%, and 20%. The treatment with *Na CMC* as a negative control, a normal control without treatment, and then the positive control was a 2% minoxidil solution treatment. The second step is to find the most effective langir bark fraction. In this second stage, the rabbit's back is drawn into 6 boxes and each smeared with water fraction, n-hexane fraction, and ethyl acetate fraction, treatment with *Na CMC* only as a negative control, a normal control without treatment, and then the positive control was 2% minoxidil solution treatment.

RESULTS AND DISCUSSION

Extraction

Langir bark was extracted using the cold maceration method. It is to prevent the chemical damage of the langir bark compound. Ethanol is the universal solvent that was able to dissolve almost all secondary metabolites compounds in the bark, non-toxic, and safely. The result of extraction of langir bark in the form of dark brown extract and distinctive smell. The yield of the extraction was measured at 10.32% w/w. The extract was then measured using a standardization *drying shrinkage* test. The result showed the 9.58% w/w value of the extract testing.

Phytochemical Screening

Phytochemical screening was performed to find out the secondary metabolite compounds present in the langir barks based on standard methods.[15,16] Table-1 shows the results of phytochemical screening of langir bark ethanol extract.

Table-1: Screening Results of Ethanol Extract of Langir Bark

Compounds	Result
Alkaloid	+
Flavonoid	+
Saponin	+
Tanin	+
Triterpenoid	+
Steroid	-
Kuinon	-
Fenol	+

Note: +: detected, -: not detected

Hair Growth *In vivo* Determination of Extract

The hair growth activity test of the langir bark extract used different doses, namely 5%, 10%, 15%, and 20%. 0.5% *Na-CMC* solution was used as a negative control, and a 2% minoxidil as a positive control because minoxidil can directly increase hair growth through the dermal papilla (DP) and epithelial cells.[17] Tests were carried out on the 3rd, 6th, 9th, 12th, 15th, 18th, 21st, and 24th days.

The activity test of langir barks extract at doses of 5%, 10%, 15%, and 20% on rabbit hair growth showed an increase in rabbit hair growth from day 3 to day 24 (Fig.-2). Table-2 and Fig.-2 showed the effective doses of rabbit hair growth. Measurement of rabbit strand growth based on the Tanaka method. The

Measurements were made every three days for 24 days, starting from days 3, 6, 9, 12, 15, 18, 21, and 24. The Measurement of the hair length was done by taking the third strand longest for each group. The hair strands were measured using tweezers and the hair length was calculated averagely for each test material. Each test material is taken with three hairs strand to determine the total hair that has grown because each rabbit fur has a different length.

Table-2: Rabbit Hair Length Measurement of Effective Dose Measurements

Treatment	Measurement of Rabbit Hair on the day to - (cm)							
	3	6	9	12	15	18	21	24
Extract 5%	0.13 ±	0.16 ±	0.22±	0.24 ±	0.27 ±	0.30 ±	0.32 ±	0.36±
	0.017	0.006	0.006	0.006	0.006	0.006	0.012	0.006
Extract 10%	0.15 ±	0.19 ±	0.24 ±	0.26 ±	0.28 ±	0.34 ±	0.37 ±	0.40 ±
	0.006	0.012	0.01	0.006	0.006	0.006	0.006	0.006
Extract 15%	0.19 ±	0.24 ±	0.26 ±	0.28 ±	0.33 ±	0.37 ±	0.40 ±	0.42 ±
	0.012	0.01	0.006	0.006	0.015	0.006	0.006	0.006
Extract 20%	0.17 ±	0.19 ±	0.21 ±	0.24 ±	0.24 ±	0.26 ±	0.31 ±	0.35 ±
	0.012	0	0.006	0.01	0.01	0.006	0.006	0.006
Positive control	0.21 ±	0.28 ±	0.32 ±	0.35 ±	0.38 ±	0.43 ±	0.47 ±	0.53 ±
	0.02	0.01	0.01	0.006	0.006	0.01	0.015	0.015
Normal control	0.00 ±	0.03 ±	0.07 ±	0.07 ±	0.07 ±	0.10 ±	0.10 ±	0.23 ±
	0	0.058	0.058	0.058	0.058	0	0	0.058
Negative control	0.03 ±	0.07 ±	0.13 ±	0.13 ±	0.13 ±	0.13 ±	0.20 ±	0.27 ±
	0.058	0.058	0.058	0.058	0.058	0.058	0.173	0.058

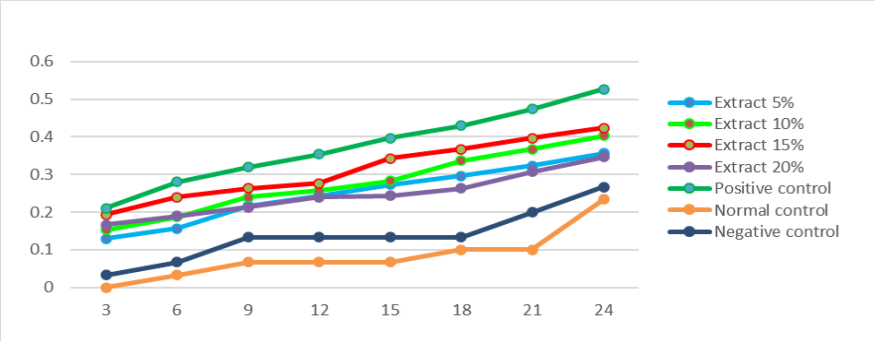


Fig.-2: Results of Rabbit Hair Length Measurement on Dose Effectiveness Test

Table-3: Results Measurement of Rabbit Hair Weight Langir Bark Extract as Hair Grower

Treatment	Measurement of rabbit hair weight (g)
Extract 5%	0.04 ± 0.01
Extract 10%	0.0567 ± 0.01
Extract 15%	0.087 ± 0.02
Extract 20%	0.067 ± 0.01
Positive control	0.097 ± 0.01
Negative control	0.05 ± 0.01
Normal	0.043 ± 0.02

The 15% dose of Langir barks extract showed the heaviest weight of 2,037 grams of the hair strand. These results are in line with the results of measurements of rabbit hair length that 15% langir bark extract showed the best results (Table-3). Hair length affects hair weight which indicated the longer and the heavier the rabbit's hair.

Hair Growth *In vivo* Determination of Fraction

Based on the extraction hair growth determination, the 15% of concentration showed good activity. So that, we use the 15% concentration for the fractions to determine the fraction hair length and weight, that resepresented in Table-4 and Table-5.

Table-4: Results Measurements of Rabbit Hair Length of Langir Bark Factions

Treatment	Measurement of Rabbit Hair on the day to- (cm)							
	3	6	9	12	15	18	21	24
n-hexane fractions	0.16±	0.25±	0.35±	0.51±	0.64±	0.78±	0.94±	1.10±
	0.005	0.005	0.020	0.005	0.015	0.026	0.02	0.025
Ethyl acetate fractions 15%	0.20±	0.29±	0.39±	0.55±	0.68±	0.82±	0.98±	1.14±
	0.005	0.005	0.002	0.026	0.020	0.036	0.015	0.015
Water fractions 15%	0.26±	0.33±	0.43±	0.59±	0.72±	0.86±	1.02±	1.18±
	0.005	0.017	0.0208	0.0230	0.0360	0.0321	0.0321	0.041
Minoxidil 2%	0.30±	0.37±	0.47±	0.63±	0.76±	0.90±	1.06±	1.22±
	0.045	0.02	0.026	0.028	0.030	0.036	0.07	0.041
Na CMC 0.5%	0.02±	0.03±	0.08±	0.18±	0.23±	0.53±	0.64±	0.74±
	0.0208	0.020	0.023	0.0208	0.036	0.0057	0.01	0.015
Normal	0.01±	0.02±	0.07±	0.16±	0.25±	0.50±	0.59±	0.63±
	0.0057	0.0208	0.023	0.020	0.0360	0.0057	0.01	0.0152

Table-5: Results Measurement of Rabbit Hair Weight Langir bark fraction

Treatment	Measurement of rabbit hair weight (g)
n-hexane fractions 15%	0.062 ± 0.028
Ethyl acetate fractions 15 %	0.063 ± 0,032
Water fractions 15%	0.086 ± 0,032
Positive control	0.090 ± 0,040
Negative control	0.050 ± 0,020
Normal	0.040 ± 0,020

The results of measurements of hair growth (Table-4) and hair weight (Table-5) of 15% water fraction showed the best hair growth compared to 15% ethyl acetate fraction and the lowest value of 15% n-hexane fraction. Meanwhile, based on the test results of 15% water fraction compared to the positive control, minoxidil showed similar values for hair weight and length. Meanwhile, based on the test results of 15% water fraction compared to the positive control, minoxidil showed similar values for hair weight and length. Hair length in 24 days from water fraction was 1.18 cm while minoxidil was 1.22 cm. Then in terms of hair weight, water fraction gave hair weight 0.086 g, while minoxidil gave hair weight 0.090. In addition, hair growth in rabbits, is represented in Fig.-3.

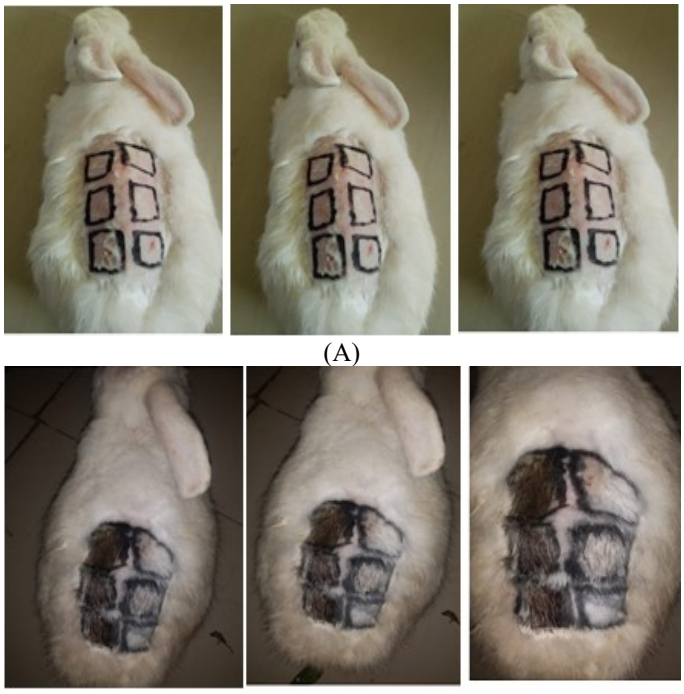
Statistical Analysis

In this study, the normality data was performed using the Kolmogorov Smirnov. Based on the Kolmogorov Smirnov data, the length of rabbit fur is usually scattered significantly p value>0.05. Furthermore, the data homogeneity test was carried out with the Levene test. Based on Levene's data, measuring the length of rabbit hair, the data showed to be homogeneous with a response significant p value>0.05. Significant differences between treatment groups were tested by one-way analysis of variance. The homogeneity data obtained a significance value >0.05, which means that the data concluded is normally distributed and homogeneous. Then the rabbit hair length data were analyzed using ANOVA and the results obtained were significance >0.05.

The ethanol extract in this research is used for the reason that ethanol solvent has the ability to extract the high polarity from nonpolar compounds to polar and sedimented proteins and inhibit the action of the enzyme so as to prevent hydrolysis and oxidation.[15] The langir barks extract showed the limit for the water compound in the extract that $\leq$ 10%, it provided that the extract can be stored for a long time without being contaminated by microorganisms in large quantities.[16]

Table-1 in the results section, showed the phytochemical screening which is conducted in this research. Ethanol extract *Albizia saponaria* bark contains showed alkaloid, flavonoid, tannin, triterpenoid, saponin, and phenol. These results were the same as those of other researchers[18]. They concluded that ethanolic extracts of root and stem barks of *Bridelia ferruginea* showed phytochemicals like alkaloids, flavonoids, glycosides, and saponins. Marsoul has reported that phytochemical screening of Punica granatum L. bark

using methanol solvents showed the presence of Phytoconstituents like phenols, alkaloids, flavonoids, tannins, and saponins (Marsoul *et al.*, 2020). In the same genus as *Albizia saponaria, Albizia lebbeck bark* has been reported the phytochemical screening of the stem bark extract contains tannins, flavonoids, saponins, phenols, and alkaloids.[20]



(A)

(B)

Fig.-3: (a) Mice at the Beginning of the Experiment; (b) Mice after Experiment

Flavonoid compounds have an activity that increases hair growth by monitoring the capillary walls of the tiny blood vessels that supply hair follicles.[10] According to Mustarichie, the secondary metabolites of waste cacao (*Theobroma cacao* L.) and Erythrina variegata can significantly increase hair growth.[3,21] Flavonoid silibinin[22] promotes proliferation and hair induction through the AKT and Wnt/β-catenin signaling pathways in 3D DP spheroids and flavonoid visnadin triggers vascular endothelial growth factor (VEGF) release.[23] In this research, hair growth stimulant activity was carried out at different levels of polarity, namely the water, n-hexane, and ethyl acetate fraction. The dose using 15% is based on the activity test results of the stem bark extract (Fig.-2, Table-3). The test animals were used male rabbits because the physiological conditions of male rabbits are relatively more stable when compared to the affected female rabbit's hormonal factors such as the menstrual cycle and pregnancy. The result of the fraction of the bark (*Albizia saponaria*) is able to grow hair and thicken the hair strands in the rabbit. It can occur because of the secondary metabolites found in the bark of the *Albizia saponaria*. The results are similar to other studies conducted by Mustarichie[3]. He found that *Erythrina variegata* extract and its water fraction contain secondary metabolites of alkaloids, saponins, flavonoids, tannins, polyphenols, and triterpenoids which are effective for hair growth in rabbits. Based on literature studies and existing publications on this plant compound saponins[24], polyphenols, terpenoids[25], and tannins[26] all the substances of the plant have compounds on increase hair growth. Statistical analysis is used to find out that the data we have is describing the real situation. First of all, basic analysis is carried out, primarily, the normality data from the hair growth activity test between the fractions of n-hexane, ethyl acetate, and water, normally distributed. Thus, from statistical analysis using ANOVA, it was identified that there are no differences between several treatments on hair growth stimulation activities effectively.

CONCLUSION

The concentration of hair growth used was 15% ethanol extract *Albizia saponaria* compounds and the water fraction was effectively increasing the hair growth of male rabbits. As a suggestion, more research should be carried out to determine which compounds are responsible for hair growth activity.

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