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Anti-Alopecia Activity of DADAP (Erythrina variegata L.) Leaves Ethanol Extract

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Abstract

Objective: This study aims to determine and verify the use of ethanol extract of Erythrina variegata leaves ethanol extract as a stimulant of hair growth or anti-alopecia.

Methods: The E.variegate leaves were collected from Salawu tribe, Tasikmalaya, West Java. The extraction was done by maceration which was based on the standard method of Indonesian Herbal Pharmacopeia. The viscous concentrated extract was fractionated by liquid-liquid extraction. Phytochemical screening according to the Farnsworth method. Method of hair growth on Angora type rabbit was modified of Tanaka method.

Results: The results of phytochemical screening using Farnsworth method showed the ethanol extract containing secondary metabolite of tannin compound, polyphenol, steroid, triterpenoid, quinone, monoterpenoid and sesquiterpenoid. The results of hair fertilizer testing using Tanaka method showed that ethanol extract and water extract with 20%, 15%, 10% concentration significantly could fertilize hair with a test for 18 days. Extracts of ethanol with levels greater than 10% showed better results. Water fraction of 10% appeared to show the best result for rabbit hair growth overcome minoxidil.

Conclusions: This work found that the concentration of 10% ethanol extract of Erythrina variegata and its water fractions were effective for hair growth on male rabbits. It was suggested, however, for further study to determine the compound which was responsible by using elucidation methods.

Keywords: Hair fertilization, ethanol extract, Erythrina variegata, hair grower, Alopecia, phytochemical screening

INTRODUCTION

Baldness is normally known as the phenomenon of aging but is associated with many other factors [1] namely, endocrine abnormalities, genetic predisposition, systemic disease, drugs, physiological abnormalities, infections, autoimmune and hair structure damage [2]. The use of hair cosmetics such as hair coloring, hair straightening, and hair curlers that too often can affect the health, strength, and fertility of hair so that one cause of hair loss over time can be severe even baldness. As many as 95% of users of hair straightener in the United States and 53% of users in Africa reported damage or hair loss due to hair straightener [3]. In addition, the habit of binding and braiding hair can also cause baldness or alopecia [4]. One of the late chemical drugs proven effective as baldness therapy is the minoxidil solution. However, minoxidil has a side effect of exfoliation during the first 4 months causing skin discomfort [5]. To avoid these side effects, herbal ingredients from medicinal plants can be an alternative solution to chemical medicine.

One of the plants empirically used by the Salawu tribe, Tasikmalaya as a hair grower is dadap leaf (E. variegate) [6]. Different parts of the E.variegata have been reported in traditional medicine as a nervine sedative, collyrium in ophthalmia, antiasthmatic, antiepileptic, antiseptic, antibacterial and as an astringent., anti-inflammatory and analgesic activity as well as anthelmintic activity and antiobesity [7-10], but has not seen any article about its efficacy as anti-baldness. This research reports activity test of ethanol extract of E.variegata leaf as a stimulant of hair growth in order to find alternative treatments against alopecia.

METHODS

Experimental animals

The experimental animal used was white male Anggora strain, aged 4-5 months, obtained from Faculty of Animal Husbandry, Universitas Padjadajaran, Bandung. Ethical clearance for animal research was applied to Health Research Ethics Committee, Medical Faculty, Universitas Padjadjaran.

Extraction method

The extraction method used was maceration with ethanol 96 %, referring to standard textbooks [11,12], for 3x24 hours. During soaking done stirring and solvent replacement several times so that the compound contained in dadap leaves could be more soluble, then filtered. The obtained macerate was concentrated with a rotary evaporator followed by heating in a water bath to obtain a thick leaf-thickened extract. This extract was then fractionated to obtain water, n-hexane, ethyl acetate fractions.

Phytochemical Screening

Phytochemical screening tests based on the Farnsworth method [13] were performed to examine the presence of alkaloids, polyphenols, tannins, flavonoids, monoterpenoids, sesquiterpenoids, steroids, triterpenoids, quinones, saponins in simplicia which could provide hair growth activities.

Alkaloid Test: Sample added 2 N 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 and observed the formation of brownish orange, and the third part was used

to be reacted with the Mayer reagent and the presence or absence of white deposits was observed.

Flavonoid Test: A few milliliters 5 N hydrochloric acid mixed with Mg metal were added to a small amount of the simplicia and then heated. The formation of red filtrate that could be drawn by amyl alcohol shows the presence of flavonoids.

Saponin Test: The sample was mixed with water in the reaction tube and then heated and then filtered. After cooling, the filtrate inside the test tube was shaken approximately 30 seconds. The presence of saponins was indicated by the formation of a 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.

Test Tannins: In the test tube containing the sample added a water amount and then heated and filtered in hot conditions. The obtained filtrate was dropped by 1% gelatin solution. The formation of white and sediment indicated the presence of tannins in the simplicia.

Polyphenol Test: A small sample was given a certain amount of water in the test tube, then the tube was heated and filtered in hot conditions. The obtained filtrate was dropped by iron (III) chloride solution. The presence of polyphenol compounds was indicated by the formation of black and blue.

Monoterpene and Sesquiterpene Test: Several samples were extracted with ether, then evaporated using a vaporizer plate to dry. In the residue was dropped reagents vanillin sulfate. Monoterpenoid and sesquiterpenoid compounds were shown by the formation of colors.

Steroid and Triterpene Test: The sample was extracted with ether, then evaporated using a vaporizer plate to dry. On the residue dripped Lieberman Burchard reagent. The formation of purple color indicated that in the simplicia contained triterpenoid group compound, whereas if formed a blue-green color indicated the presence of steroid group compounds.

Quinone Test: The sample was heated in water, then filtered. Then the filtrate formed in the NaOH. The yellow to red color formed showed the quinone content in the simplicia.

Testing Hair Growth Activity

Testing of hair growth activity was done on ethanol extract of E.variegata leaves. The method used was based on a method of Tanaka *et.al*, [14] which had been used and modified by others [15-18]. In this research using one method of hair fertilizer activity that was shaving method, back of hair rabbit was shaved to clean then divided into 7 boxes with size 2x2 cm. The experiment steps were as follows,

Test Animal Preparation: Rabbits used were 4 male rabbits, calculated using Federer rule [19], 4-5 months old, no anatomical defects and no pain. Prior to use, rabbits were acclimatized for 7 days in order to familiarize themselves with the environment and new treatment. Then the back was cleaned from the feathers and rested for 24 hours.

Dilution Extracts: Preparation of dadap leaves extract concentrations of 20%,15%,10% , and 5%. The preparation of each concentration was carried out by weighing 500 mg NaCMC, sprinkled over hot water in a mortar, left until fluffy, stirring vigorously until completely blended and then adding the extract, then mixing until homogeneous and then adding aquadest to 100 mL.

Rabbit Grouping, A total of 4 male rabbits were used. Rabbits were shaved back and divided into 7 boxes measuring 2x2 cm and given the following treatment. The test was carried out in two stages, the first stage of the test looked for the effective dose in which the rabbit's back was divided into 7 parts and each was smeared with 2% minoxidil solution as a positive control, treated with NaCMC only as a negative control, no treatment as a normal control, dadap leaves extract of 5%,10%,15% and 20%. The second stage was to find the most effective of fractions. For this, of the rabbit's back was divided into 6 squares and each smeared with 2% minoxidil solution as a positive control, treated with NaCMC only as a negative control, no treatment as a normal control, water fraction, n-hexane fraction, and ethyl acetate fraction.

Data analysis

After the data obtained from the results of research, then performed data processing using Statistical Analysis of Variance (ANOVA). The ANOVA is a collection of statistical models used to analyze the differences between group means and their associated procedures [20].

RESULTS AND DISCUSSION

Extraction

Maceration is a technique used in medicinal plants research [21]. Dadap leaves were extracted by cold maceration method. Cold maceration method was used to prevent the occurrence of chemical damage to dadap leaves content. The ethanol solvent was chosen because ethanol was a universal solvent which was able to dissolve almost all secondary metabolites contained in the leaf in a non-toxic and safe manner. With the ability to extract a wide polarity from nonpolar compounds to polar and precipitate proteins and inhibit the action of the enzyme so as to avoid hydrolysis and oxidation was another reason why ethanol was chosen [22]. The macerate which was collected from the maceration process was then concentrated by using a rotary evaporator at a low pressure and a temperature of 40 ^{0}C to obtain a viscous extract and to avoid the destruction of secondary metabolite compounds due to overheating. Heating was carried out till a constant weight. The result of extraction of dadap leaves in the form of brown extract and distinctive smell. The yield of the extraction was 14.96 % w/w. The result of the determination of water content of this extract was 3.88 % w/w, which means it did not cross the limit of the deep water content which stated the water content in the extract should be ≤ 10% [11,12]. In other words, the extract could be stored for a long time with not contaminated by microbes or fungi in a significant amount.

Phytochemical screening results

Phytochemical screening was performed based on standard methods [13, 22, 23] to find out the secondary metabolite compounds present in the dadap leaf. Table 1 shows the results of phytochemical screening of dadap leaves ethanol extract.

Table 1. Screening results of ethanol extract of dadap leaves

Compounds	Results
Alkaloids	+
Flavonoids	+
Tannin	+
Polyphenols	+
Saponin	+
Steroids	+
Quinone	-
Monoterpenoid dan sesquiterpenoid	+

Note: +: detected, -: not detected

Table 1 showed the results of the procedures undertaken in this study. These results were similar to those of other researchers including Subrhamanian *et.al* [24] concluded that E.variegate flower possessed phytochemicals like alkaloids, flavonoids, glycosides, saponins and steroids, Muthukrishnan *et.al* [25] reported that phytochemical screening of E.variegata leaves extract in five different solvents showed the presence of important phytoconstituents like phenols, alkaloids, flavonoids, tannins and saponins, Kumari *et.al* stated their phytoconstituents like phenols, alkaloids, flavonoids, tannins, and saponins were detected in their sample. From existing publications the plant compounds such as polyphenols [27], 2000) , terpenoids [28], alkaloids [29], flavonoids [30], tannins [31], saponins [32], steroids [33], and quinone [34], all of these substances in plants that have properties on hair growth. It looked like what was more dominant and came from what plant.

Hair growth activities results

The Most Active Dose Effectiveness of dadap leaves extract

The effectiveness test of active fraction dose was carried out to the dadap sample extract 5 %, 10 %, 15 %, and 20 %. Preparation of test substances was done by weighing as much as 500 mg Na CMC and developed till form mucilage. This gel was then used in determining the most effective dose in fertilizing hair growth. The test was carried out for 18 days with 0.5 mL smearing every morning and afternoon. The drawing of the plastering can be seen in Figure 1.

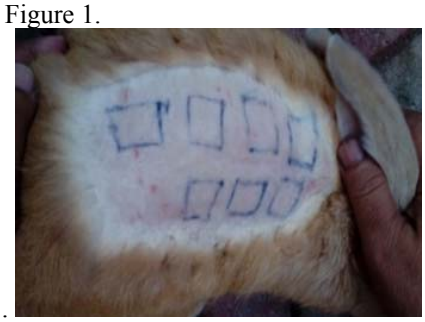


Figure 1. Sketch on the back of a rabbit

Table 2 and Figure 2 represent the rabbit hair length measurement of effective dose measurements.

Measurement of the growth of rabbit hair was based on Tanaka method which was carried out for 18 days with the timeframe taken every three days. Measurements were made by taking the longest five hairs from each test material. Then calculated using a caliper and calculated the average hair length of each test material. Taking 5 pieces of each test material was done so that the results obtained would represent the total hair that growth because each rabbit hair had a different length. Besides, by taking 5 pieces of each box (test material) was expected to lose due to the removal would be avoided. Based on the above Table 2 the growth of rabbit hair was quite visible at the beginning of the measurement but had not shown any significant difference. The low growth in negative control compared with normal control because of the negative control, the carrier material of NaCMC possibly cover the pores of the skin thus blocking the growth of hair. In the 18th saw the best growth of rabbit hair was on the extract with a concentration of 10% with a length of 0.89 cm. On the measurement before the 12th day, there was no noticeable change, after which a measurable difference was seen. This result was in line with our previous research which also used the Tanaka method but used the roots of Angiopteris evecta L.[16].

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
Normal control	0,33 ± 0,086	0,37 ± 0,093	0,61 ± 0,162	0,73 ± 0,164	0,79 ± 0,172	0,84 ± 0,121
Negative control	0,34 ± 0,138	0,40 ± 0,252	0,60 ± 0,238	0,70 ± 0,242	0,78 ± 0,233	0,82 ± 0,284
Positive control	0,32 ± 0,161	0,40 ± 0,199	0,62 ± 0,254	0,73 ± 0,303	0,83 ± 0,340	0,86 ± 0,319
Extract 5%	0,34 ± 0,144	0,40 ± 0,199	0,80 ± 0,219	0,73 ± 0,225	0,78 ± 0,170	0,82 ± 0,144
Extract 10%	0,32 ± 0,112	0,42 ± 0,170	0,68 ± 0,289	0,74 ± 0,214	0,84 ± 0,216	0,89 ± 0,085
Extract 15%	0,33 ± 0,020	0,39 ± 0,093	0,68 ± 0,118	0,74 ± 0,133	0,83 ± 0,137	0,82 ± 0,107
Extract 20%	0,32 ± 0,078	0,38 ± 0,132	0,68 ± 0,104	0,70 ± 0,075	0,82 ± 0,074	0,86 ± 0,131

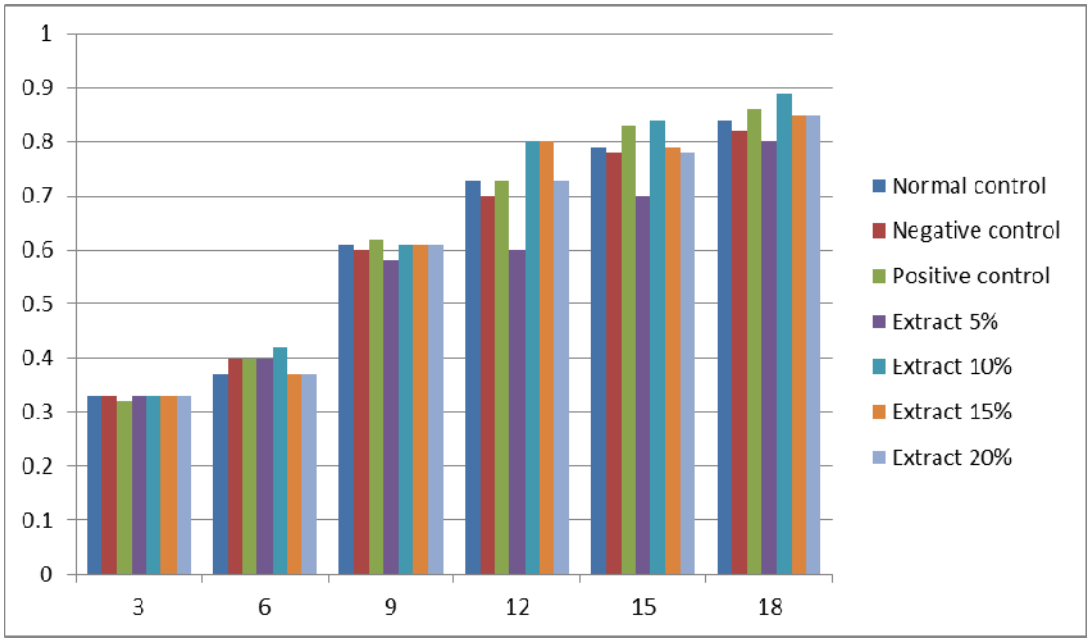


Figure 2. Results of Rabbit Hair length measurement on Dose Effectiveness test

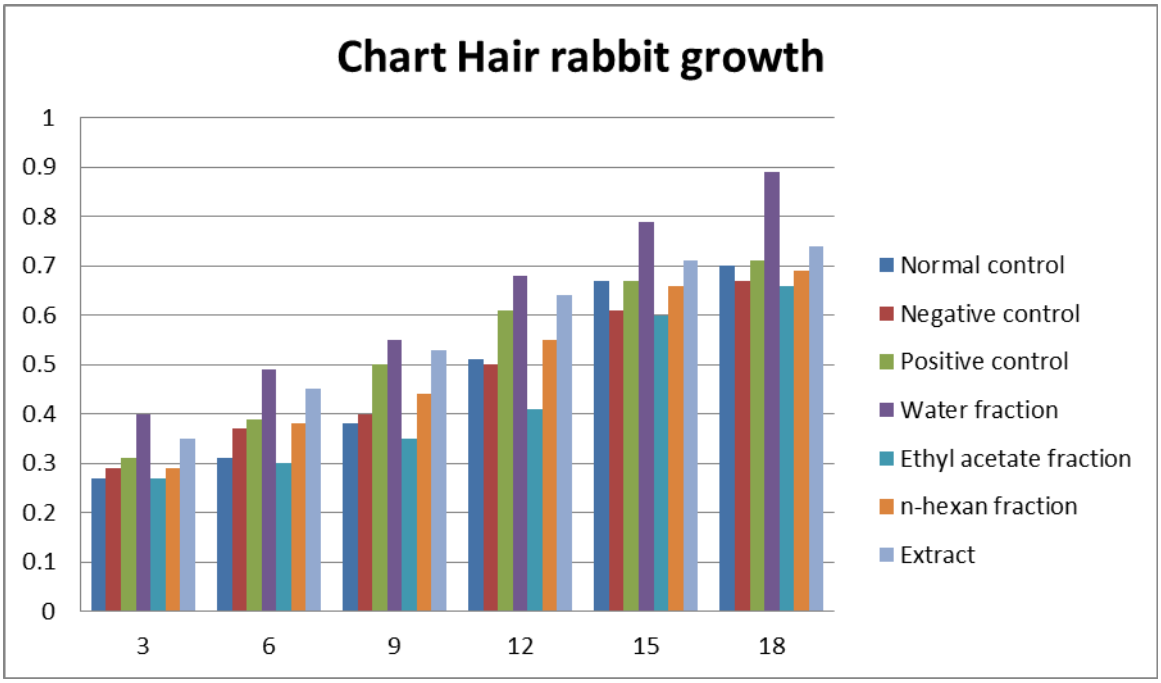
Table 3 Results measurements of rabbit hair length of water, ethyl acetate and n-hexane factions

Treatment	Measurement of rabbit hair on the day to - (cm)					
	3	6	9	12	15	18
Nomal control	0,27	0,31	0,38	0,51	0,67	0,70
SD	± 0,075	± 0,025	± 0,045	± 0,015	± 0,067	± 0,056
Negative control	0,29	0,37	0,40	0,50	0,61	0,67
SD	± 0,071	± 0,115	± 0,076	± 0,071	± 0,017	± 0,026
Positive control	0,31	0,39	0,50	0,61	0,67	0,71
SD	± 0,058	± 0,042	± 0,006	± 0,036	± 0,031	± 0,017
n-hexan fraction	0,29	0,38	0,44	0,55	0,66	0,69
SD	± 0,044	± 0,092	± 0,083	± 0,081	± 0,056	± 0,045
Ethylacetate fraction	0,27	0,30	0,35	0,41	0,60	0,66
SD	± 0,035	± 0,049	± 0,021	± 0,035	± 0,120	± 0,085
	0,42	0,54	0,59	0,61	0,79	0,95
Fraksi air	0,32	0,35	0,4	0,65	0,75	0,86
	0,45	0,57	0,65	0,77	0,83	0,87
Rata-rata	0,40	0,49	0,55	0,68	0,79	0,89
SD	± 0,068	± 0,119	± 0,131	± 0,083	± 0,040	± 0,049
Extract	0,35	0,45	0,53	0,64	0,71	0,74
SD	± 0,070	± 0,046	± 0,084	± 0,118	± 0,038	± 0,030

Examination of active fraction of dadap leaves extract

In this research, testing of stimulant activity of hair growth was carried out for water, n-hexane and ethyl acetate fractions. The concentration used in this study was 10% which was based on the results of Figure 2. Selection of male rabbits as the animal test in this study because the male rabbit physiological conditions were relatively more stable when compared with female rabbits affected by the hormonal factor such as menstrual cycle and pregnancy. Meanwhile, rabbits aged 4-5 months in general because rabbits aged 4-5 months had entered into the adult category and physiological conditions and anatomy had been perfect. Before the treatment was given, the rabbits were acclimatized first for 7 days so that the rabbit could adapt to the condition of the surrounding environment so that the potential stress would be reduced. On day 6 of acclimatization, the rabbit was shaved on the back then smeared 70% alcohol as an antiseptic, after which the rabbit was rested for 24 hours. Next on the 7th day the back of the rabbit that has been shaved given a sign of a box measuring 2x2 cm with a distance of 1 cm each box. The use of a distance of 1 cm each box serves to prevent mixed fractions with each other, so the results obtained remain based on the ability of each faction to be tested.

From table 3 illustrated in Figure 3, it can be seen that at the end of the 18th-day treatment it is seen that the water fraction from leaves extract is the best fraction for hair growth. Many of the plants on sale are said to be effective as alopecia, such as Aloe vera, ginseng, rosemary, Indian gooseberry, Neem, Sage, curry leaves, holy basil, soap nuts, ginger, hibiscus flower [35] but none of others, which states which fractions are efficacious and at their concentration can be used.



Statistic analysis

To know that the data we have describes the real situation then the data needs to be analyzed by using statistical analysis. In the experiment times, the activity of stimulating hair growth between n-hexane, ethyl acetate, and water fractions was done by looking at normality test of data. The normality test was the most basic statistical analysis prior to further statistical analysis. Normality test here served to see whether the data we got followed or close to the normal distribution or not. In this research, the data normality test was by using Kolmogorov Smirnov test. Based on Kolmogorov test Smirnov data length of normally distributed rabbit hair with significance value> 0.05. Further tested the homogeneity of data with Levene test. Based on the Levene test, rabbit hair length measurement data was said to be homogeneous with significant value for response> 0,05. With the fulfillment of normality and homogeneity of this data, to know the presence or absence of significant differences among the treatment group was tested by one-way variance analysis. Prior to the ANOVA test, rabbit hair length data were tested for its homogeneity. From the data of homogeneity of significance value obtained> 0,05 which meant the data obtained normal distribution and homogeneous. Then the data length of rabbit hair was analyzed by using ANOVA and obtained the result significance> 0.05 which meant there was no difference in the influence of some treatments to stimulate hair growth activity

CONCLUSIONS AND SUGGESTIONS

It was found that the concentration of 10% ethanol extracts of Erythrina variegata and its water fractions were effective for hair growth on male rabbits. It was recommended, however, for further study to determine the compound which was responsible for it by using structure elucidation method.

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