



RESEARCH ARTICLE

Formulation of Hair Tonic of Meniran (*Phyllanthus niruri* L.) Ethanol Extract as Hair Grower in Male White Rat (*Rattus norvegicus*) Wistar Strain

Sri Luliana, Rise Desnita, Rani Rawinda*

Dept. of Pharmacy, Medical Faculty, Tanjungpura University, Pontianak, Indonesia.

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ABSTRACT

Meniran (*Phyllanthus niruri* L.) has potential as a hair grower. Hair loss problems often found in various ages, both women and men. The purpose of this study was to determine the effectiveness of tonic preparations *P. niruri* ethanol extract as a hair grower. Tonic preparations made using 5% ethanol extract of *P. niruri* and additives such as ethanol 96%, propylene glycol, menthol, DMDM hydantoin, sodium metabisulfite, and TEA. Tonic preparations applied topically to the skin that has been shaved backs of mice. Observations carried out on hair length on day 7, 14 and 21, while the weight of the hair on the 21st day. Primary skin irritation test performed on 18 animals back skin test.

KEYWORDS

P. niruri L., Hair tonic, Hair growth activity

INTRODUCTION

The loss is one of the problems on the hair that can affect the appearance, both physical and psychological disorders through hair loss is not a disorder that can be life threatening^{1,2}. Patient data in RSUPN Dr. Cipto Mangunkusumo stated that during 2009 to 2011 as much as 39.7% alopecia aerata and 34.5% had effluvium telogen³. One cosmetic product developed to treat hair loss is hair tonic. Hair tonic preparation is a cosmetic preparation that is used on the scalp to stimulate hair growth in hair loss or baldness⁴. The active ingredient of hair tonic when it was developed from natural materials. One of the plants that have potential as a hair grower is meniran (*Phyllanthus niruri* L.). *P. niruri* containing lignans and terpenoids

can increase the activity of hair growth through the mechanism of inhibition of 5 α -reductase enzyme activity⁵. *P. niruri* extract hair tonic made to be easily applied to the scalp. In addition, the hair tonic has a trans-appendageal absorption is good, not greasy and leaves no residue on the scalp compared to others liquid or semi-solid preparation⁶. The ability of *P. niruri* ethanol extract hair tonic as hair growth can be enhanced by using penetrant enhancers. The penetrant enhancer is a compound that can help the process of absorption in the skin through a reduction in skin impermeability⁷. One of the penetrant enhancer used in this study is menthol. The aim of this study was to examine the effectiveness of hair tonic preparations from *P. niruri* ethanol extract as a hair grower in Wistar male rats.

MATERIALS AND METHODS

The tools used in this study is a rotary evaporator (Buchi R II), caliper (tricle Brand), pH meter (Hanna), GF₂₅₄ TLC plate (E Merck), scales digital (Ohaus Type PA 2012) and glassware. / Herbs Herbal Natural Grace, Yogyakarta. Other

***Address for Correspondence:**

Rani Rawinda,
Dept. of Pharmacy,
Medical Faculty, Tanjungpura University,
Pontianak-78124,
Indonesia.

materials used include quercetin is (Sigma Aldrich), simplicia *P. niruri* used herbs obtained from Supplier and Distributor of Materials Herbal, cream depilatories (Veet®), minoxidil (Regrou®), ethanol 96%, propylene glycol, sodium metabisulfite, menthol, DMDM hydantoin, TEA, and water. Test animals used were male Wistar rats at the age of 2-3 months and a weight of 150-250 grams. The population of test animals comes from Bantul. Animal tests used have passed the review of conduct, with number 739 / UN22.9 / DL / 2018.

Extraction

The extraction is done using the maceration method with a solution of ethanol 70%. A total of 1.128 kg of *P. niruri* powder was added with ethanol 70% while occasionally stirring. 24 hours later filtered and the residue was maceration until the filtrate obtained clear by using total ethanol 70% 16.5 L. The filtrate obtained is collected, then the solvent evaporated using a rotary evaporator at a temperature of ± 40°C to obtain a thick extract, then calculated extract yield.

Standardization Extract of Specific Parameters

Phytochemical Screening and Thin Layer Chromatography

Standardization extract of specific parameters done of phytochemical screening and TLC. TLC identification was performed using GF₂₅₄ silica plate with quercetin standard. The extract was dissolved in methanol and spotting appearance used is AlCl₃ 5%.

Determination of Total Flavonoid Content

The method used is the Chang method. The standard solution is made by weighing 25 mg of quercetin dissolved in methanol and then diluted to 1000 ug/ml. Then made a series of concentration 24; 21; 18; 15; 12 and 9 ug/ml from standard stock solution. 2 ml of each series of concentration added with 0.1 ml AlCl₃ 10%, 0.1 ml CH₃COONa 1 M, and 2.8 ml of distilled water. The mixture is shaken until homogeneous and allowed to react for 30 minutes at room temperature before being a measured absorbance

at λ 434 nm. Standard curve equation is then used to measure the levels of total flavonoid extract ethanol *P. niruri*. Total flavonoid content (TFC) were then calculated using the following equation⁸.

$$TFC = \frac{R \times D.F \times V \times 100}{W}$$

Where:

R: The results obtained from the standard curve

D.F: Dilution factor

V: The volume of stock solution

W: Weight of the sample used

Hair Tonic Preparation Formulations

Hair tonic preparations in this study were made in two formulations with a concentration of extract that is used by 5% (w / w) in the same base composition, wherein one of the formulas use penetrant enhancer menthol. Positive control used in the form of tonic preparations containing minoxidil 2%.

Table 1: The Composition of the Materials Used in the Manufacture of Tonic

No.	Material	Concentration (%)	
		F1	F2
1	<i>P. niruri</i> ethanol extract	5	5
2	Menthol	-	1
3	Ethanol	30	30
4	Propylene glycol	2	2
5	Sodium metabisulfite	0.1	0.1
6	DMDM hydantoin	0.5	0.5
7	TEA	0.5	0.5
8	Water	Add 100	Add 100

Making the Tonic Preparations

Tonic preparations of *P. niruri* ethanol extract made by weighing the ingredients needed. The extract is then dissolved with warm water before adding ethanol, while menthol was dissolved with ethanol sufficiently. Sodium metabisulfite, TEA and DMDM hydantoin each dissolved in water. Combine parts of water and ethanol slowly, propylene glycol was added little by little and the remains of the water while stirring until homogeneous.

Evaluation of Tonic Preparations

Tonic preparations were evaluated organoleptically and pH test. Organoleptic testing was conducted on the color and smell of the preparation. Meanwhile, preparations pH testing is done by using a pH meter. pH meter calibrated beforehand using a buffer solution with a pH of 9. After calibration, the pH meter is dipped into the preparation and left for several minutes to obtain a pH of tonic preparation.

Primary Skin Irritation Test

18 male white rats Wistar strain adaptation for 7 days, were divided into 3 groups: group 1 as F1, group 2 as F2 and group 3 as control, then placed each one in a cage. The hair on the backs of the rat was shaved using a razor on 3 different places, then smeared 0.5 g cream depilatory (Veet®) for 5 minutes and rinsed with water. Then made a box as a test area with a size of 2 x 2 cm. 24 hours later skin irritation test was performed⁹.

0.5 ml test preparation is applied to the three test areas each treatment group. The test area was closed using sterile gauze and plaster. Observations were made at the 24th, 48th and 72nd hour after treatment the test preparation. After 24 hours, plaster and gauze opened (area 1: located on the upper back), as well as the existing residue, is washed with water prior to observing. Observations made 48 hours in area 2 which is at the center of the back and observations made 72 hours in area 3 located in the lower back. Observations were carried out with the parameters of erythema and edema⁹. The response of the test preparation is then calculated

to obtain the skin primary irritation index by Primary Irritation Index (PII)¹⁰.

$$PII = \frac{A - B}{C}$$

Where:

- A: Total score of erythema and edema throughout the observation point samples at the 24th, 48th and 72nd hour divided by the number of observations
- B: Total score of erythema and edema throughout the observation point control at the 24th, 48th and 72nd hour divided by the number of observations
- C: Number of animals

Primary irritation index values obtained are then used to determine the level of irritation as the following table¹⁰.

Table 2: Categories Irritation Response¹⁰

Average value	Category Response
0,0 – 0,4	Very light (negligible)
0,5 – 1,9	Mild irritant (slight)
2,0 – 4,9	Irritant (moderate)
5,0 – 8,0	Strong irritant (severe)

Hair Grower Activity Test

0.5 ml test preparation applied to the backs of mice were shaved once a day. Prior to application of the test preparation, alcohol 70% is applied to the test area that has been shaved as an antiseptic. The first day basting regarded as day 0. Observations hair grower activities carried out for 21 days. A total of 6 strands of longest hair of mice removed using tweezers on day 7, 14 and 21. Hair then straightened out and placed on selotip. Hair length is measured using a caliper. Measurements were also conducted on hair weight mice on day 21 by way of a haircut that grows in the test area and weighed for each

group. The results were then analyzed statistically.

Data Analysis

The results of measuring the length of the hair of mice were analyzed using SPSS Statistics 22 to determine whether there are significant differences in the speed of hair growth of mice on day 7, 14, and 21 as well as the weight of hair of mice on the 21st day of testing, both in the control group and the test group. Data tested normal distribution and homogeneity of its variants ($p > 0.05$), then the data is tested with One-Way ANOVA and Post Hoc LSD with 95% confidence level.

RESULTS AND DISCUSSION

Extraction and Standardization of Extract

Extraction using maceration method and obtained viscous extract as much as 284.14 g with a value of yield 25.18%. The viscous extract obtained is then performed the phytochemical screening. Phytochemical screening results showed that *P. niruri* ethanol extracts contain secondary metabolites alkaloids, flavonoids, phenols, tannins, terpenoids/steroids, and saponins. These results are consistent with previous research which stated that *P. niruri* contains tannins, steroids, alkaloids, and flavonoids¹¹.

In addition, thin layer chromatography profiles using spotting appearance $AlCl_3$ produces a yellow color indicating the presence of flavonoids, can be seen in Figure 1. One of the flavonoids contained in *P. niruri* is quercetin¹². Quercetin in the *P. niruri* ethanol extract was measured using the Chang method. The result of the measurement of total flavonoid content was $2.01\% \pm 0.15$, in accordance with the requirements of Indonesian Herbal Pharmacopoeia, not less than 0.90% calculated as quercetin¹³.

Mobile phase = Chloroform: Methanol: Water (80: 12: 2) to flavonoids, quercetin standard.

Note: 1 = *P. niruri* ethanol extract, 2 = standard quercetin. (A): Observations UV 254 nm; (B):

Observations UV 366 nm; (C): after being sprayed reagent $AlCl_3$ 5%.

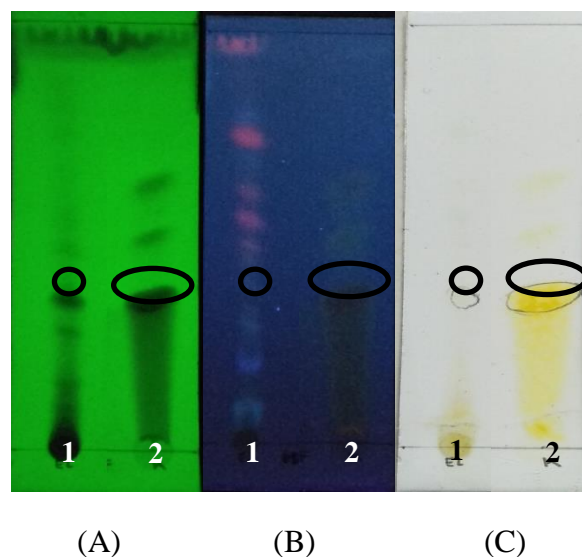


Figure: 1 Profile of Thin Layer Chromatography

Hair Tonic of *P. niruri* Ethanol Extract and Evaluation Preparations

Tonic preparations were made because it is easier to use, not sticky and does not leave a thin layer on the skin such as the use of another semisolid dosage. Tonic preparations prepared by dissolving the extract with warm water and then added with 96% ethanol. In addition, ethanol is also used as a solvent menthol. Propylene glycol is used as cosolvent of extract so that it is completely dissolved and the ingredients used in the formulation. DMDM hydantoin is used as a preservative because of the content of water in large quantities can be a microbial growth medium. Sodium metabisulfite is used as an antioxidant to prevent oxidation of the *P. niruri* extract. Menthol is used as penetration enhancers to the skin, giving it the smell of fresh and cool sensation on the scalp.

Tonic preparations made were evaluated organoleptically and pH. The evaluation results are shown in Table 3. Observations organoleptic of both the tonic formula shows that the preparations are not transparent. This is because *P. niruri* extracts used in the form of dark viscous extract thus resulting preparation becomes dark, which is colored black. pH of

tonic preparations in the range pH of the skin that is 4-7 and meet the quality requirements of BSN 16-4955-1998 stating that tonic preparations pH in the range 3-7^{14,15}.

Table 3: The Results of Evaluation of *P. niruri* Ethanol Extract Hair Tonic

No.	Evaluation	Result	
		F1	F2
1	Appearance	Black, typical aroma	Black, typical aroma of menthol
2	pH ± SD	6 ± 0.01	5.94 ± 0.01

The Primary Skin Irritation Test

The primary irritation test was aimed to observe the effects that arise in the skin irritation test animals. The principle of this irritation test is the provision of the test preparation in a single dose to the skin of test animals with such control untreated skin. Observations were carried out at the 24th, 48th and 72nd hour with the parameters of the presence or absence of erythema and edema¹⁰. Tonic preparations irritation testing results in table 4 show that the observations at 24 hours, 48 hours and 72 hours after administration dosage scoring erythema and edema zero so that it can be concluded that the preparation tonic formula 1 and formula 2 is not irritating to the skin of test animals.

Test Activity Hair Grower

P. niruri contain secondary metabolites that could potentially stimulate hair growth. One of them is quercetin. Based on previous studies, quercetin can prevent hair loss in test animals. One mechanism that is as an anti-inflammatory by inhibiting HSP70¹⁶. Moreover, the results indicate that *P. niruri* identification of compounds containing phenols. Phenols compounds in *P. niruri* can be lignans, which are methylated phenols. Lignans role in inhibiting the activity of the 5 α -reductase enzyme in order to reduce the conversion of testosterone into dihydrotestosterone potentially cause hair loss⁵.

Parameter tests conducted to test the activity of hair growth is the average length and weight of

rat hair. Hair length measurements can be used to express the tonic preparations ability to stimulate the growth of hair length mice while weighing the hair is used to determine the effect of tonic preparations against hair luxuriance mice.

Based on the chart above, it is known that a group of the commission of Formula 1, Formula 2 and negative control mice resulted in hair length greater than the normal control. This may be due to additional materials such as ethanol 96%, propylene glycol, and water. Ethanol and propylene glycol can decrease the skin impermeability to water making it easier for water to pass through the stratum corneum. Ethanol and propylene glycol can change the permeability of the skin through interactions with the hydrophilic group on the lipid bilayer which can certainly increase the dosage partition into the stratum corneum¹⁷.

Formula 2 gives results of hair length measurements that are greater than the formula 1, negative control and normal control on 21st-day observation. This could be due to the use of enhancers menthol concentration of 1%. Based on the structure is, menthol is a terpene class of chemical enhancers. The use of enhancers class of terpenes and simultaneously propylene glycol having work synergistically so that it has a better ability enhancers¹⁸. Menthol relative has a shorter interval than the other terpenes enhancer group resulting in a relatively short time has given effect¹⁹.

The treatment group formula 1 and 2, when compared to the positive control minoxidil, gives the average hair length is smaller. Minoxidil can increase hair follicle size of values into the terminal and maintain terminal hair to prevent hair loss. In addition, minoxidil is also known to increase DNA synthesis in the anagen phase hair root so that it can stimulate the growth of the secondary from telogen follicles to produce rapid growth into the anagen follicles²⁰.

Table: 4 Observations Primary Skin Irritation of Tonic *P. niruri* Ethanol Extract

No.	Preparations	The Observation / Value Score					
		24 hours		48 hours		72 hours	
		Erythema	Edema	Erythema	Edema	Erythema	Edema
1	F1	0	0	0	0	0	0
2	F2	0	0	0	0	0	0
3	Control	0	0	0	0	0	0

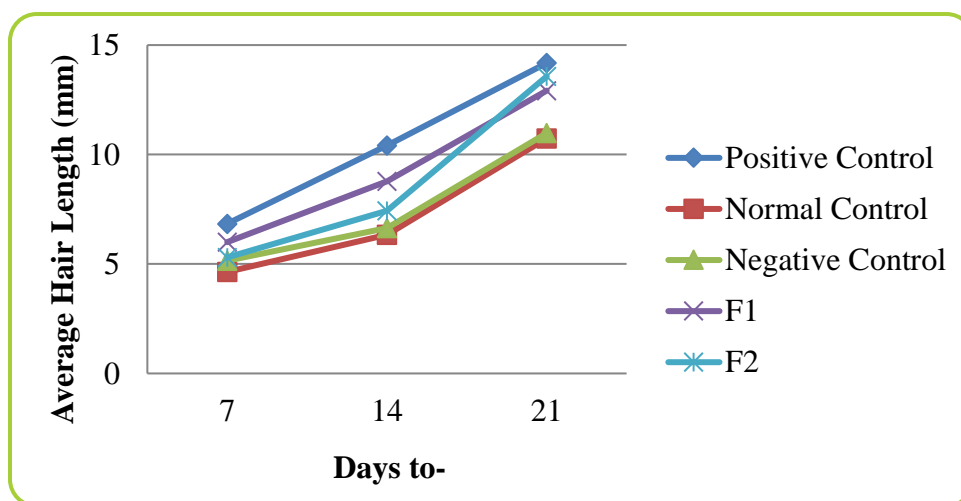


Figure: 2 Graph Average Hair Length Mice on Day 7, 14 and 21

Table 5: The Result of the Calculation of the Average Length and Weight Hair of Each Group Treated

Group	Treatment	The average hair length (mm) ± SD			The average weight of the hair (mg) ± SD
		day 7	day 14	day 21	
1	Control (+)	6.84 ± 0.58	9.87 ± 1.81	14:18 ± 2.76	33.56 ± 25.09
2	Normal control	4.64 ± 0.66	6:06 ± 0:47	10.74 ± 1:59	2.67 ± 1.99
3	Control (-)	5:15 ± 0.96 #	7:08 ± 0.99 #	10.98 ± 1:12	10:43 ± 0:15
4	F1	6:00 ± 1:47 *	9:15 ± 1.88 *	12.92 ± 3:47	23.97 ± 15:59
5	F2	5:30 ± 0.63 #	7.82 ± 1.61	13:57 ± 2.88	26.47 ± 10.95

Description: (*) Different significant (p <0.05) to normal control.

(#) Different significant (p <0.05) to positive control.

Hair growth rats at day 7 clearly visible yet. However, on further observation is day 14 and 21, an increase in the growth of mouse hair length. Testing the growth of hair length mice on day 7, 14 and 21 respectively using statistics show normally distributed data and homogeneous so proceed with ANOVA test. The test results using ANOVA states that on day 21 there were no significant differences between the treatment groups. This shows that the group of formula 1 *P. niruri* ethanol extract tonic preparations have equivalent activity hair regrowth formula 2. However, based on the results of measurements of the average length of the hair, the formula 2 containing menthol 1% penetrant enhancers have a greater value than the formula 1.

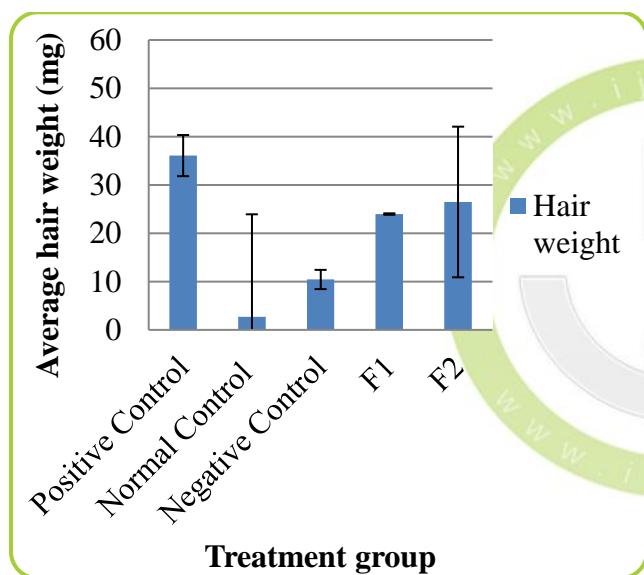


Figure: 3 Graph the Average Hair Weight on Day 21

Hair weight observations made on the 21st day. Hair each test area of each treatment was shaved and weighed. Based on data from the results of weighing the hair weight, formula 2 has a greater weight than the other treatment groups. Statistical analysis showed that the weight of the data was normally distributed and homogeneous hair so it continued with the ANOVA test. The test results using ANOVA states that there is no significant difference in weight groups of mice. This suggests that the formula 1 preparation may stimulate flashes of hair tonic which is

equivalent to formula 2, although not statistically significant differences.

CONCLUSION

Based on this study it can be concluded that the formula 2 dosage tonic of *P. niruri* ethanol extract containing penetrant enhancer menthol 1% have an activity as a hair grower a better comparison with formula 1, although statistically, the formula does not show any significant difference both in terms of length or hair weight.

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