

Development and Evaluation of Waru (*Hibiscus tiliaceus* Linn.) Leaf and Avocado (*Persea americana* Mill.) Fruit Extracts for Hair Growth

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Abstract—Hair tonic formulation of waru (*Hibiscus tiliaceus* Linn.) leaf and avocado (*Persea americana* Mill.) fruit in various concentrations were studied for their physical stability, hair growth activity, potential of irritation, and rating of hedonic. The waru leaves contains saponin as counter-irritant, flavonoid as bacterisid to prevent the hair fall by the bacteria, and tannin as the protein protector in hair compiler. The avocado fruit contains vitamin E as hair conditioner. The extract of waru leaves and avocado fruit were prepared in varying concentration in the formulation of hair tonic by water preparation technique. Physical stability were tested by cycling test method observed from organoleptic, pH, viscosity, and homogeneity parameters. Hair growth activity were tested on the rabbits (*Oryctolagus cuniculus*) hair. Potency of irritation were tested by spreading on arm skin of probandus. Rating of hedonic were tested by questionnaire by panelists. Physical stability test result showed that all formulas have good stability unless formula which contained 4% of waru leaves extract and 8% of avocado fruit extract. Hair growth activity test resulted that formula which contained 3% of waru leaves extract and 8% of avocado fruit extract had obtained as the best formula. Potency of irritation test result showed that all formulas have no irritation potentially. And the rating of hedonic test result showed the most preferred formula which contained 4% of waru leaves extract and 4% of avocado fruit extract. The studies concluded that waru leaves and avocado fruit can formulated to hair tonic as hair growth.

Keywords— Hair tonic, Waru, Avocado, Formulation, Stability, Hair growth, Irritation, Hedonic.

I. INTRODUCTION

Hair is one of the body considered to protective appendages on the body and accessory structure of the integument along with sebaceous glands, sweat glands and nails. At the present time hair loss is common problem in men and women due to excessive exposure of chemicals in daily routine on scalp [1]. Researchers innovate to find effective hair growth formula using active substances include natural ingredients. One of preparation is a hair tonic as a hair care preparations used for the prevention and treatment of baldness or other ailments, aggression of hair. They also promote the luxurious growth of hairs [2]. Hair tonic used after shampooing on the scalp surface until the liquid was absorbed.

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The active substance in hair tonic have different effects, namely as counter irritant, vasodilators, stimulants of sebaceous glands, hair conditioners, hormones, and antiseptics. The active substance can have only one effect or combination of several effects. The natural ingredients derived from plants developed as the primary substance used in beauty and health care [3].

Natural ingredients that are empirically used as fertilizer hair one of which is a leaf of waru (*Hibiscus tiliaceus* Linn.) which contains saponins, flavonoids, and polyphenols [4] – [5]. Flavonoid can prevent free radicals and accelerate the hair growth. Saponin can increase blood flow to the hair follicles so that nourish and accelerate the hair growth. Polyphenols and tannins can bind and protect the proteins needed for hair growth. [6]

Other natural ingredients are also empirically believed as hair growth is fruit of avocado (*Persea americana* Mill.) which contains protein and vitamin A, B, B1, B2, E and C, beta-carotene, lenolic acid, lecithin, calcium, iron, pantoneat acid, potassium, phosphorus, niacin, and natural plant oil [7]. Its contents effect as a conditioner that promote hair growth.

Rakmawati (2008) formulated the ethanol extract of waru leaves into ointment has the activity of hair growth [8]. Indriwinarni (2011) formulated the ethanol extract of waru leaves into a gel has activity of hair growth in mice [9].

Therefore waru leaf extract and avocado fruit can be formulated in a stable hair tonic proven to have hair growth activity in rabbits (*Oryctolagus cuniculus*). The preparation then evaluated its safety by irritation potential testing on human skin. Then hair tonic will know the level of consumer interest in the product by hedonic test. Expected hair tonic produced can be developed into products with high effectiveness and efficacy.

II. PROCEDURE

A. Ingredients

Ammonia, acetic acid, avocado fruits, butanol (Merck), chloroform (Merck), distilled water, ethanol, filter paper, waru leaves, iron (III) chloride, gauze, menthol, methanol, methyl paraben, propyl paraben, sodium metabisulfite, reagent of Dragendorff, reagent of Mayer, sulfuric acid, propylene glycol, X brand of hair tonic.

B. Tools

Rotary vacuum evaporator (Buchi®), furnace (Carbolite®), waterbath (Biocote®), beaker (Pyrex®), pipette, analytical balance (Precisa®), filler (Super Orior®), measuring pipette (MC®), volumetric flask (Pyrex®), rod stirrer, pH-meter (Jenway®), syringe, Ostwald viscometer (AceGlass®), porcelain dish (Pyrex®), pycnometer (Pyrex®), electric cutter (Miyako®), calipers, hair clippers, scissors, sheet of questionnaires.

C. Sample processing

Waru leaves was obtained in Muna, Southeast Sulawesi, then washed, cut, dried, powdered to obtain the powdered of simplicia. Avocado fruits obtained in Kolaka, Southeast Sulawesi, then washed, the fruit was taken, and smoothed.

D. Extraction

Each simplicia were macerated with ethanol, filtrates were evaporated using rotary vacuum evaporator then extracts were stored in desiccators and used for subsequent experiments.

E. Formulation of hair tonic

TABLE I
FORMULAS OF HAIR TONIC

Ingredients	Concentration (%)(W/V)						
	K	F1	F2	F3	F4	F5	F6
Waru leaves extract	-	2,00	2,00	3,00	3,00	4,00	4,00
Avocado fruits extract	-	4,00	8,00	4,00	8,00	4,00	8,00
Ethanol	30,00	30,00	30,00	30,00	30,00	30,00	30,00
Propylene glycol	15,00	15,00	15,00	15,00	15,00	15,00	15,00
Sodium metabisulfite	0,01	0,01	0,01	0,01	0,01	0,01	0,01
Propyl Paraben	0,01	0,01	0,01	0,01	0,01	0,01	0,01
Methyl Paraben	0,1	0,1	0,1	0,1	0,1	0,1	0,1
Menthol	0,30	0,30	0,30	0,30	0,30	0,30	0,30
Distilled water	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100

Extract of waru leaf and avocado fruit was dissolved in propylene glycol. Then propyl paraben, methyl paraben, menthol, and sodium metabisulfite was dissolved in ethanol. Then phase propylene glycol and ethanol were mixed, up volume to 50 ml with distilled water, and put in a sealed container.

F. Stability Test of Hair Tonic

Conducted by cycling test. Hair tonic stored at 5°C and 40°C respectively 24 hours (one cycle). The treatment repeated for 6 cycles. The physical and chemical condition of hair tonic was compared before and after treatment.

Organoleptic: Hair tonic was observed about changes in color, scent, and homogeneity.

Value of pH: Hair tonic of 20 ml was measured by pH-meter through constant number listed as pH value

Viscosity: Hair tonic of 10 mL was inserted through the tube and hair tonic sucked up through the lower and upper bounds past. The hair tonic allowed to flow from upper limit to lower limit. The time required for the hair tonic to flow measured by stopwatch. The viscosity then calculated using the formula:

$$\frac{\eta_1}{\eta_2} = \frac{\rho_1 t_1}{\rho_2 t_2} \quad (1)$$

η_1 = viscosity of hair tonic

η_2 = viscosity of water

ρ_1 and ρ_2 = the specific gravity of each liquid

t_1 and t_2 = length of flow (second)

G. Hair Growth Activity Test

The rabbits were acclimatized for 5 days and divided into 3 replication of one rabbit respectively 4 cm² area of surface of each rabbits shaved area to remove the hairs. Approximately 9 areas was applied a hair tonic of 0.5 mL once a that was continued for 15 days. During the course the hair growth initiation pattern was observed and reported. [10] Observations of long hairs in each area was did on 6th, 12th, and 18th days. Total of four strands of hair rabbits in each test area was measured using a caliper.

H. Primary Skin Irritation Test

Performed on 21 panelists. Hair tonic of 2,5 ml was to the gauze 2,5 x 2,5 cm² which covered by aluminum foil, then glued on the upper arm of panelist and allowed to stand for 24 hours. Furthermore, the gauzes were opened and rinsed by water. The tes sites were observed for erythema and edema for 40, 48, and 72 hours after application. [11]

TABLE II
POTENCY OF IRRITATION

Category of Edema	Value of Edema	Category of Erythema	Value of Erythema
No edema	0	No erythema	0
Very mild edema	1	Slight erythema	1
Mild edema (edges and clear enlargement)	2	Erythema apparent	2
Moderate edema (thickness ± 1 mm)	3	moderate to strong erythema (30-35 cm)	3
Severe edema (thickness ± 1 mm)	4	Severe erythema (35-40 mm)	4

I. Hedonic Test

Panelists consisted of 25 men and 25 women. Each panelist chose the formula of the stocks that are considered attractive hair tonic according to taste. Panelists fill out a questionnaire. Then calculated the percentage of preference for each hair tonic. [12].

III. RESULTS

A. Formulation of Hair Tonic

Making of hair tonic was used some excipients. Ethanol used as solvent of methyl paraben, propyl paraben, and menthol. Propilen glycol used as solubility enhancers of extracts and as humectants. Menthol used as penetration enhancers to the skin and fresh sensation. Methyl paraben and propyl paraben used as preservatives. Sodium metabisulfite is used as antioxidant.



Fig. 1 Hair Tonic

B. Stability Test

Stability test was conducted by cycling test in order to accelerate the change in normal condition ie the decomposition of materials. Consisting of observation organoleptic, homogeneity, viscosity, and pH values.

Organoleptic observations done to obtain the physical changes (color and scent) of hair tonic. Table 2 shows that all the hair tonic formula does not distort color and aroma of hair tonic before and after cycling test. This was in accordance with the Standar Nasional Indonesia (SNI), which remains liquid [13]

TABLE III
OBSERVATION OF ORGANOLEPTIC

Formula	Cycling Test	Form	Color	Scent
1	Before	Liquid	Greenish brown	Menthol
	After	Liquid	Greenish brown	Menthol
2	Before	Liquid	Greenish brown	Menthol
	After	Liquid	Greenish brown	Menthol
3	Before	Liquid	Brown	Menthol
	After	Liquid	Brown	Menthol
4	Before	Liquid	Brown	Menthol
	After	Liquid	Brown	Menthol
5	Before	Liquid	Dark brown	Menthol
	After	Liquid	Dark brown	Menthol
6	Before	Liquid	Dark brown	Menthol
	After	Liquid	Dark brown	Menthol

Six formula has different color. The higher concentration of the extract was added, the darker color of hair tonic was resulted.

Homogeneity testing performed by visually observing the particles were not soluble and precipitate before and after cycling test. Table 4 shows that there is a formula which was experiencing the deposition after the cycling test is formula 6.

TABLE IV
OBSERVATION OF HOMOGENITY

Formula	Homogeneity	
	Before cycling test	After cycling test
1	without sediment	without sediment
2	without sediment	without sediment
3	without sediment	without sediment
4	without sediment	without sediment
5	without sediment	without sediment
6	without sediment	with sediment

Sedimentation of formula 6 was due to the high concentration of extracts so that the evaporation that occurs when cycling test leads to reduced solubility of the extract in a hair tonic. Unless formula 6, all formulas have good stability. This was in accordance with the Standar Nasional Indonesia (SNI) that there was no particles or sediment [13].

The pH value can affect the effectiveness, stability, and comfort of use of transdermal dosage form. The alkaline will cause skin feels sleek, dry, and affect the elasticity of the skin. But the acidic leads easily irritated skin [14]

TABLE VI
DATA OF LENGTH OF RABBIT HAIR

No.	Length of rabbit hair on 12th day (cm)										Length of rabbit hair on 18th day (cm)								
	F1	F2	F3	F4	F5	F6	K ⁺	K ⁻	KN	F1	F2	F3	F4	F5	F6	K ⁺	K ⁻	KN	
1	1.3	1.38	1.44	1.52	1.4	1.69	1.57	1.29	1.29	1.42	1.73	1.98	2.12	1.76	1.85	2.12	1.39	1.29	
2	1.3	1.34	1.40	1.52	1.37	1.45	1.55	1.3	1.29	1.39	1.74	1.99	2.09	1.75	1.89	2.1	1.4	1.29	
3	1.34	1.33	1.43	1.54	1.36	1.44	1.59	1.29	1.27	1.38	1.76	2.02	2.11	1.77	1.87	2.14	1.41	1.27	
4	1.32	1.36	1.42	1.56	1.35	1.45	1.6	1.28	1.28	1.41	1.78	2.01	2.1	1.75	1.85	2.15	1.4	1.28	
n	1.32	1.35	1.42	1.53	1.37	1.51	1.58	1.29	1.28	1.4	1.75	2	2.10	1.76	1.87	2.12	1.4	1.28	

TABLE V
OBSERVATION OF pH VALUE

Formula	pH	
	Before Cycling test	After cycling test
1	6,15	6,21
2	6,12	6,19
3	6,13	6,20
4	6,12	6,24
5	6,00	6,17
6	5,97	6,16

The higher the concentration of extract, the lower the pH value of hair tonic because the extract is acidic. After cycling test all the formulas increased pH due to degradation due to temperature changes extracts. All formulas have good stability. This was in accordance with the Standar Nasional Indonesia (SNI), which a range of pH 3-7 [13].

Hair tonic which has a high viscosity will leave the crust in the head so that trigger dandruff. Viscosity values vary according to the concentration of propylene glycol and extracts were added. the higher the concentration of extract the higher the viscosity of hair tonic.

TABLE VI
OBSERVATION OF VISCOSITY

Formula	Viscosity (cps)	
	Before cycling test	After cycling test
1	1,63	1,69
2	1,68	1,80
3	1,68	1,83
4	1,84	1,95
5	1,81	2,07
6	1,90	2,2

The results indicated all formulas has increased viscosity because the solvents evaporated during cycling test and extract compounds and excipient degraded. Viscosity increasing did not affect the stability of the preparations because it was within the range of viscosity grades. This was in accordance with the Standar Nasional Indonesia (SNI), which below 5 cps at room temperature (25 °C) [13].

C. Hair Growth Activity Test

Shaved hair of rabbits has not grown up to the 6th day, it was suspected because rabbits are traumatized and are still adapting to shaving. treatment of rabbit hair begins to grow on the 2nd week. Measurements were taken at 12th day.

Normality test shows that the long-hair rabbits in each treatment area has a normal distribution (Sig. ≥ 0.05). Homogeneity test showed that the length of the hair rabbit homogeneous (Sig. ≥ 0.05).

Anova of length rabbit hair on 12th day shows that there are significant differences (real different) hair length data between treatment areas (sig. ≤ 0.05) at 12th day. This means that all formulas had different hair grower activity.

LSD test was performed to determine the formula that has best activity by comparing one with another formula. The results showed that the formula 4 (3% waru leaf extract and meat extract 8% of an avocado) have the most activity is good but not equivalent to the positive control (sig ≤ 0.05).

Normality test shows that the long-hair rabbits in each treatment area has a normal distribution (Sig. ≥ 0.05). Homogeneity test showed that the length of the hair rabbit homogeneous (Sig. ≥ 0.05).

Anova of length rabbit hair on 18th day shows that there are significant differences (different real) hair length data between treatment areas (sig. ≤ 0.05) on the 18th day. This means that all formula hair tonic activity hair grower different.

LSD test was performed to determine the formula that has best activity by comparing one with another formula. The results showed that the formula 4 (3% waru leaf extract and meat extract 8% of an avocado) have the most activity is good but not equivalent to the positive control (sig ≤ 0.05).

Formula 4 had better activity than the formula 5 (4% waru leaf extract and 4% avocado fruit extract) and the formula 6 (4% waru leaf extract and 8% avocado fruit extract). Formula 5 and 6 contains extracts of waru leaves in high concentration. Waru leaves contain saponins that affect counter irritant. The high concentration of waru leaf extract causes damage to the cells of the scalp. As a result, the nutrients used to repair the hair cells of the scalp so that hair growth is not maximal.

Waru leaves contains flavonoid and polyphenols that act as antioxidants to reduce free radicals [15] and as a bactericidal and antivirus to inhibit bacteria and viruses so as to accelerate growth and prevent hair loss [16]. Saponins also be counter irritant resulting in increased peripheral blood circulation thereby increasing hair growth [17]. Polyphenols and tannins can bind and protect the proteins needed for hair growth [6].

D. Irritation Test

Irritation test was conducted to determine the potential for irritation and the security level of the preparation of hair tonic various concentrations that have been formulated. Irritation test conducted on 21 female panellists aged 20-30 years old, able-bodied, and has signed a letter of approval and is ready to bear the responsibility for any consequences that will occur during the test and would not prosecute as contained in the attachment.

Upper inner arm area selected as an area of test because the stratum corneum was thin so that absorption of the sample will large, sheltered place to not be easily separated, and if irritation occurs will not be visible from the outside.

Based on the results irritation based on primary irritation index of 0 is included in the category by no means. This was showed that the saponins in the waru leaves and excipients

that used in concentrations that are still safe and did not cause irritation. So that formula of hair tonic was safe in use.

E. Hedonic Test

Hedonic test was performed by measuring the level of preference by using a questionnaire sheet by 50 panelists aged 20-50 years. Assessment undertaken to see the colors and scents of hair tonic preparations with varying concentrations of extract of waru leaf extract and avocado fruit with coding every preparation to use three random numbers to minimize the subjectivity of assessment.

TABLE VII
RESULTS OF HEDONIC TEST

Formula	Percentage of hedonic (%)		
	Like	Neutral	Dislike
1	2 4	44	32
2	4 2	38	20
3	8 8	6	6
4	4 6	42	12
5	1 2	12	76
6	6	34	60

IV. CONCLUSION

Waru leaf and avocado fruit extracts can be formulated and developed to hair tonic as hair growth.

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