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Short Communication

HAIR TONIC FORMULATION OF CLOVE LEAVES (*SYZYGIUM AROMATICUM*) ETHANOL EXTRACT AND THE EFFECTIVENESS ON RABBIT HAIR GROWTH

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ABSTRACT

Objective: The purpose of this study was to determine the physical stability and effectiveness of hair growth in hair tonic preparations containing ethanol extract of clove leaves (*Syzygium aromaticum*) with a concentration variation of 5, 10, and 15%.

Methods: Formulations were evaluated, including organoleptic observation, pH checking, and preparation stability tests using the Climatic Chamber. The effectiveness of clove leaf extract as a hair tonic was tested by looking at hair growth in test animals.

Results: The results showed that the preparation was stable at high temperatures (40±2 °C) where the odor, color, clarity, and pH remained constant. Effectiveness test on rabbit hair growth showed significantly different results after measuring hair growth on the 21st day, where the results were 11.83+0.06 mm for positive control, 1.71+0.06 mm as blank, 1.69+0.06 mm for the group without treatment, and successively the results of the group given clove leaf extract 5, 10, and 15% were 4.00+0.06; 4.80+0.06; and 5.46+0.08 mm.

Conclusion: Hair tonics containing clove ethanol extract can increase rabbit hair growth.

Keywords: Ethanol extract, Hair tonic, *Syzygium aromaticum*

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Hair loss is a condition where the hair is detached from the surface of the skin, such as the head and body. Head loss can occur physiologically or pathologically, which is influenced by external and internal factors in the body. In general, hair loss can be caused by heredity and the influence of hormones. It also can be caused by a lack of nutrient intake into the hair, free radicals, side effects of drugs, stress, and an unhealthy diet [1–3].

One way to prevent hair loss is by doing hair care. In treating hair needs a variety of cosmetics, ranging from hair cleaning cosmetics, hair conditioner, cream bath, and hair tonic. An easy way to treat hair loss is to do hair care using hair tonic as an ingredient to nourish hair [4–7].

Hair growth stimulants (hair tonic) are preparations that contain ingredients needed by hair, hair roots and scalp [1, 2]. Currently, hair tonic preparations are widely available in the market both from chemicals and herbal ingredients [8, 9]. The use of chemicals in cosmetics products is considered less safe because it can cause side effects on long-term use. One of the synthetic chemicals commonly used in hair tonic as a nutritious agent is Minoxidil which has allergic skin side effects, headaches, vertigo, weakness and edema [10–12].

The clove (*Syzygium aromaticum*) belongs to the Myrtaceae family and is one of the native Indonesian spice plants [13]. Clove is one type of spice plant that has high antioxidant activity due to the high content of eugenol. Clove is a spice plant that has long been used in the clove cigarette industry, food, beverages and medicines. Parts of plants that can be utilized are flowers, flower stalks and clove leaves [14–16].

The antioxidant activity of *Syzygium aromaticum* leaf extract is quite good. The highest total antioxidant activity and reducing power were obtained from methanol extract and both were closely related to the total phenol content of clove leaves [17, 18]. Based on the results of TLC analysis it is estimated that the compounds contained in clove leaves are alkaloids, phenolics, flavonoids, triterpenoids, terpenoids and saponins. Saponin, phenolic and flavanoid compounds in each plant have a role to stimulate hair growth [19-21].

Empirically the people of Nias Island Indonesia, use clove leaves to trigger hair growth by using clove leaf oil which is applied to the scalp regularly morning and evening. In this article we will discuss the activity of clove leaf extract in triggering hair growth or as a hair tonic.

The tools used in this study include rabbit maintenance equipment (rabbit cages, feed containers, and drinking containers), shoves to measure the length of rabbit hair, analytical balance, scissors and razor blades, pH meters, drop pipettes, spatulas, tools glassware, and extraction equipment (blenders, filter paper, funnels, rotary evaporators).

The clove leaf (*Syzygium aromaticum*) was obtained from the island of Nias, Indonesia. The solvent used was 96% ethanol, and the other chemicals were sodium metabisulfite, methyl paraben, propyl paraben, menthol, tween 80, propylene glycol and distilled water.

The clove leaves were washed with running water. Clove leaves are dried in a drying cabinet to form a simplicia. Simplisia clove leaves are mashed using a blender then extracted using maceration method using 96% ethanol solvent. Maceration is done for 5 d. Then maserat is filtered using filter paper to produce filtrate and residue. The resulting residue was remaserated for 2 d while stirring occasionally. Then the filtrate from the results of the first maceration and remaseration was collected and concentrated with a rotary evaporator until a thick extract was obtained [22, 23].

The formula used is a standard gel formula that is modified with the addition of the extract as the active substance. The formula used in making hair tonic preparations (24) can be seen in table 1.

Dissolve tween 80 in a portion of distilled water (solution 1), then dissolve the extract in solution no. 1 (solution 2). Dissolve sodium metabisulfite in distilled water (saolution 3). Dissolve each ingredient of nipagin, nipasol and menthol in ethanol then mix with solution No. 3 (solution 4). Add propylene glycol into solution 4 while stirring homogeneously (solution 5). Mix solutions 2 and 5, add distilled water to the volume limit.

Table 1: Hair tonic formula

No.	Chemicals	F0	F1	F2	F3
1.	Clove Leaf Extract (%)	-	5	10%	15%
2.	Ethanol 96% (ml)	30.00	30.00	30.00	30.00
3.	Sodium Metabisulfite (g)	0.01	0.01	0.01	0.01
4.	Methylparaben (g)	0.25	0.25	0.25	0.25
5.	Propylparaben (g)	0.025	0.025	0.025	0.025
6.	Menthol (g)	0.10	0.10	0.10	0.10
7.	Tween 80 (ml)	2.0	4.0	6.0	8.0
8.	Propylene glycol (ml)	15.00	15.00	15.00	15.00
9.	Distilled Water up to (ml)	100	100	100	100

Evaluation of the formula includes organoleptic observation, pH checking, and preparation stability test using the Climatic Chamber. Hair tonic is observed organoleptic changes, including: consistency, odor and color [25, 26]. The pH was measured by means of a pH meter. The instrument is first calibrated using a neutral pH standard buffer solution (pH 7.01) and an acidic pH buffer solution (pH 4.01) until the instrument shows the pH value, the electrodes are washed with distilled water, then dried with tissue paper. Then the electrode is dipped in a hair tonic preparation solution, until the instrument shows a constant pH value. The number shown by the pH meter is the pH value of the formula [25, 26]. Physical stability test was carried out by storing hair tonic at 40±2 °C using Climatic Chamber. Organoleptic observations and pH measurements were carried out once a week for 1 mo [27].

Animals that are used as research objects are rabbits (*Oryctolagus cuniculus*). Before the research began, rabbits were acclimated for 2 w so that the rabbits could adapt to their new environment. During the acclimation process, rabbits are given standard feed. Before testing all the rabbits to be used shaved hair on the back first. Then a depilatory cream is applied so that the hair roots are thoroughly clean. After that, 6 treatment boxes were made with each box area of 4 cm2 (2 cm x 2 cm) and each box was spaced 1 cm apart. The treatment box is demarcated using permanent markers to differentiate between the locations of the treatments [28, 29]. The illustration can be seen in fig. 1.

Hair tonic is applied to each treatment box on the back of a rabbit as much as 1 ml twice a day for 3 w. Determination of the length of hair that grows on the back of the rabbit after being shaved is done on the 7th, 14th and 21st days. Pull hair up to the root of the hair using tweezers. A total of 10 longest rabbit hair lengths were measured using calipers. Data on average hair length obtained were processed statistically to see whether there were significant differences between the test areas and positive control [30].

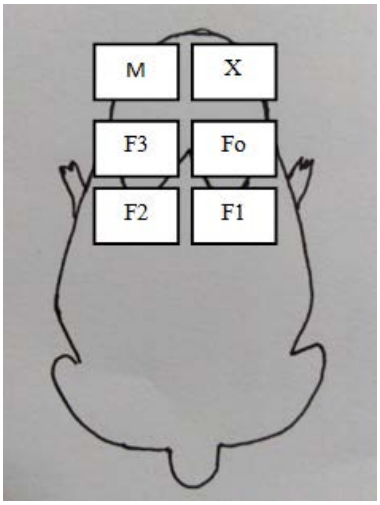

Fig. 1: Illustration of the location of tonic formula hair tonic on rabbit, Explanation-M: Positive Control, X: Without Treatment, F0: Negative Control (Blank), F1: Clove Leaf Extract 5%, F2: Clove Leaf Extract 10%, F3: Clove Leaf Extract 15%

Fig. 2: Clove leaves extract hair tonic

Determination of hair weights is done to determine the effectiveness of hair tonic preparations against hair dense. Hair weight measurement is done after 21 d by shaving the hair that grows in the test area then weighed. The results obtained were calculated statistically to see whether there were significant differences between the test areas and positive controls [24, 30].

Hair tonic with the addition of clove leaf extract, each 5% light brown, 10% dark brown, and 15% blackish brown. Clove hair tonic preparations of clove leaves have a distinctive clove odor. Hair tonic F0 (blank) is transparent (clear) and has a distinctive smell of menthol. The formulated hair tonic can be seen in fig. 2.

The results of checking the pH value on hair tonic are still in the range of pH values that do not irritate the skin (pH balance). The pH value obtained is 4.5-6.0 where the desired pH range is 4.5-6.5. So it can be concluded that each formula has the characteristics of a good pH value. The pH value of hair tonic can be seen in table 2.

Table 2: pH value of hair tonics

Hair tonic	Average of pH
F0	6.00±0.057
F1	4.70±0.057
F2	4.60±0.057
F3	4.60±0.057

The Data given in mean+SD, number of each formula (F0-F3) was 3 times of test.

Hair tonic preparations stored in a Climatic chamber with a high temperature of 400C±20C for 2 w did not show any changes in shape, color, or aroma. This shows that the preparation is stable. The pH check is one of the testing parameters to determine whether the preparation is included in the skin's pH range or not, which is 4.5-6.5. Where the pH of the preparations used can affect absorption of the skin. If the pH of the preparation is too acidic, it can cause skin irritation. And if the pH of the preparation is too alkaline it can cause scaly skin [31, 32].

Based on the results of measurements of rabbit hair length for 21 d, each concentration of the formula has effectiveness on the growth of rabbit hair. The results of hair growth in rabbits can be seen in table 3.

Table 3: The results of hair growth

Groups	Hair growth value		
	Day-7	Day-14	Day-21
Positive Control	0	6.35±0.042	11.83±0.06
Formula 1 (5%)	0	2.27±0.056	4.00±0.06
Formula 2 (10%)	0	2.86±0.048	4.80±0.06
Formula 3 (15%)	0	3.16±0.042	5.46±0.08
Formula 0 (Blank)	0	0±0	1.71±0.06
Without Treatment (X)	0	0±0	1.69±0.06

The Data given in mean+SD with number of each formula (F0-F3) was 3 times of test.

Based on the fig. of the statistical test results, each concentration of the clove leaf formula showed results that were further lower than the positive control, the formula containing Minoxidil 2%. This is influenced by the mechanism of action of minoxidil in stimulating hair growth by extending the anagen phase and increasing the size of hair follicles. Histology shows that minoxidil therapy can increase the proportion of hair follicles in the anagen phase and decrease hair follicles in the telogen phase [33].

Hair growth is influenced by compounds contained in clove leaves, including phenolic, flavonoids, and saponins. Saponin, phenolic and flavonoid compounds in each plant have a role to stimulate hair growth. Flavonoid compounds are found in many plant tissues which act as antioxidants. Phenolic compounds in leaves are also responsible for antioxidant activity. Saponin compounds are secondary metabolites produced by mainly dicotyledonous plants. Saponins in the human body function to increase blood flow to the hair follicles, if blood flow to the hair follicles decreases it will affect the hair follicles and cause hair loss [29, 34-36].

The results of this study concluded that hair tonic clove leaf ethanol extract showed relatively good physical stability, where the high temperature stability test of odor, color, clarity, and pH still showed constant results so that the preparation was safe for use. Hair tonic extract of clove leaf ethanol can increase rabbit hair growth.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

No conflict of interest associated with this work. All authors guarantee that the contents of this article are known and approved by all parties

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