

HAIR TONIC OF BROCCOLI (*BRASSICA OLERACEA* VAR. *ITALICA*) EXTRACT: FORMULATION, PHYSICAL CHARACTERISTIC, STABILITY AND HAIR GROWTH ACTIVITY TEST

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ABSTRACT

Objective: Hair loss is a common scalp concern for both men and women. Broccoli (*Brassica oleracea* var. *Italica*) is a vegetable plant in the cabbage family or brassicaceae that has antioxidant activity and levels of flavonoids that can encourage hair growth. Hair tonic is one of cosmetic products that contain ingredients to nourish hair. The purpose of this study was to investigate the physical stability and activity of hair development in hair tonic preparations using broccoli stalks extracted at different concentrations of 30%, 35%, and 40%, followed by an examination of the hair growth activity test in an animal model.

Methods: This study hair tonic was prepared with Broccoli (*Brassica oleracea* var. *Italica*), the sample used the broccoli stalks, which was taken by purposive sampling. Evaluation of the preparation includes physical characteristic observation, pH examination, homogeneity of the preparation, irritation test, and stability test of the preparation using a cycling test, and check the activity test of hair growth in rats' skin.

Results: The results showed that the hair tonic preparation containing broccoli stalks extracts was stable at a temperature (4 °C±40 °C) where the odor, color, shape, homogeneity, and pH remained constant. In statistical testing using ANOVA analysis, each group of the hair tonic formula of broccoli stalks extract showed hair growth activity in male white rats. The hair tonic formulation containing 40% broccoli stalks extract had better hair growth activity compared to the 30% and 35% formula groups. The hair tonic prepared from broccoli extract is confirmed safe for use after successfully passing the skin irritation test.

Conclusion: The hair tonic of broccoli extracts had met the standard of physical characteristics during storage time and it has a good activity test on hair growth in male white rats. This study provides a novel formulation in hair growth.

Keywords: Hair Tonic, Broccoli (*Brassica oleracea* var. *Italica*), Hair loss

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INTRODUCTION

Hair has a crucial role for living creatures, evident from its function as protection from adverse surroundings, including cold or hot temperatures and ultraviolet light. Human hair serves a dual purpose, functioning as both a protective barrier and a prominent aesthetic feature. Improving one's physical appearance, especially by having attractive hair, frequently results in a significant increase in an individual's self-confidence [1-3]. Adults encounter hair-related issues, one of which is alopecia. Alopecia is the reduction in the quantity of hair follicles on the scalp. This condition can be attributed to hormonal imbalances, adverse reactions to medicines, dietary factors, and psychological stress [4-6]. Hair loss is a common occurrence that can diminish one's aesthetic appeal. Shielding the body and head from external elements [7, 8]. Decreasing the amount of hair on the head can lead to psychological distress, particularly among women. If the number of strands of hair lost every day surpasses 100, hair loss is considered to be serious [9-12]. The causes of hair loss are diverse, classified as endogenous due to systemic diseases, hormonal imbalances, nutritional state, intoxication, or genetic abnormalities, and exogenous due to environmental stimuli or hair cosmetics [10, 13, 14].

Currently, there is a wide range of hair tonic solutions available in the market, derived from both natural and synthetic chemical compounds as well as herbal substances [15-17]. The primary purpose of hair tonic is to enhance blood circulation in the scalp, hence preventing hair loss, promoting hair growth, preventing dandruff and itching, and imparting a revitalizing sensation to the scalp [18-20]. According to regulations set forth by the Food and Drug Administration (FDA), hair tonic is a cosmetic product that is utilized for the purpose of

promoting hair development. The hair tonic formulation consists of solvents, helpful ingredients, vasodilators that widen blood vessels to promote hair growth, hormones (both cosmetic and pharmaceutical preparations), anti-peptic agents, and fragrances [3, 14, 21]. Broccoli, scientifically known as *Brassica oleracea* var. *Italica*, is an annual plant classified under the Brassicaceae family. It is closely related to other vegetables such as Brussels sprouts, cabbage, cauliflower, and kale. The term "broccoli" is derived from the Italian word "broccolo," which refers to the flowering head of a cabbage [22-24]. Broccoli originated in the eastern Mediterranean region and was then imported to America and England during the 17th century, and to China in the late 1900s [25]. Broccoli (*Brassica oleracea* var. *Italica*) is a plant that is often chosen to be combined with other fiber-rich plants due to its high content of vitamins and minerals [26, 27]. The content of vitamins includes vitamin A, C, E, K, B1, and B6, which are sufficiently high to be combined with functional fibers that can cause unavailability of nutrients such as fat-soluble vitamins [28, 29]. The broccoli stalk (*Brassica oleracea* var. *Italica*) contains chemical compounds such as flavonoids, sesquiterpenes, monoterpenes, and steroids [30]. The flavonoid present in the stalks of Broccoli (*Brassica oleracea* var. *Italica*) can stimulate hair growth by inducing muscle relaxation in the blood vessels and surrounding hair follicles [31, 32]. The residual produce of Broccoli (*Brassica oleracea* var. *Italica*) is eliminated after processing [33]. Broccoli stalks, scientifically known as *Brassica oleracea* var. *Italica* retains their fiber, vitamin C, and phenolic components. The author is intrigued by the potential advantages of the extract derived from the Broccoli stalks (*Brassica oleracea* var. *Italica*) and aims to create hair tonic formulations using this extract. The objective is to evaluate the hair growth activity in male white rat through experimentation [20, 23, 29, 30]. The broccoli stalks (*Brassica*

oleracea var. *Italica*) were used to create a solution-based hair tonic in this investigation. The hair tonic solution was selected for its ease of application and non-sticky nature, which prevents the production of crusts that may contribute to dandruff formation [34-36].

This study aimed to formulate a hair tonic containing Broccoli stalks extract (*Brassica oleracea* var. *Italica*) in various concentrations, observing its physical stability and testing its activity of hair growth on rats' skin.

MATERIALS AND METHODS

Materials

Broccoli stalk (*Brassica oleracea* var. *Italica*) obtained from North Sumatra, Indonesia with a determination certificate number: 6076/MEDA/2021 by Herbarium Medanese (MEDA) University of North Sumatra, Indonesia. Stearic acid (Sigma Aldrich, St. Louis, MO, USA), cetyl alcohol (Honeywell, UK), sorbitol (Sigma Aldrich, St. Louis, MO, USA), propylene glycol, triethanolamine (J. T Baker), methyl paraben (Sigma Aldrich, St. Louis, MO, USA), distilled water, perfume, ethanol 96% (Honeywell, UK).

Tools

The tools used in this research include a pH meter (Jenco, San Diego, USA), a rotary evaporator (Heidolph instrument), a stirrer, rat rearing equipment (rat cages, food containers and drinking containers), calipers to measure length rat hair, dropper pipette, spatula, glassware and extraction equipment (blender, filter paper, funnel).

Extraction of *Brassica oleracea* var. *Italica*

Macerate the refined heads of 3000 g of Broccoli (*Brassica oleracea* var. *Italica*) with 75 parts of 70% ethanol solvent for 5 d, during the process place them in a cool place while stirring occasionally, filter the macerate using a funnel and paper to produce a filtrate and residue. 70% ethanol until 100 parts are obtained (25 parts remaining solvent), leave for 2 d in a cool place while stirring occasionally, filter the results of the remaceration using a funnel and filter paper to produce a filtrate and residue, collect the filtrate from the results of the first maceration and remaceration, Concentrate with a rotary evaporator until a thick extract is obtained [37, 38].

Phytochemical screening

Alkaloid test

Few mg of extract was taken in 5 ml of 1.5% v/v hydrochloric acid and filtered. These filtrates were then used for testing alkaloids.

Dragendorff's test: Dragendorff's reagent was added in 2 ml of filtrate. Formation of orange-brown precipitate indicated the presence of alkaloids.

Mayer's test: To a 1 ml of test filtrate in a watch glass, a few drops of Mayer's reagent were added. If the formation of cream colored precipitate it shows the presence of alkaloids.

Flavonoid test

Add a few drops of 37% HCl and add Mg powder. The test results showed that extract was positive for flavonoids if indicated by the red color produced after the HCl reagent and Mg were added.

Terpenoid test

In a test tube containing 2 ml of chloroform, 0.5 ml of extract was added. This is then followed by the addition of 3 ml conc. H₂SO₄ which forms a layer. Reddish brown coloration of the interface indicates terpenoids.

Steroid test

Salkowski test

10 mg of extract was dissolved in 2 ml of chloroform and 2 ml of concentrated sulphuric acid was added from the side of the test tube. Test tube was shaken for a few minutes. The development of red color in the chloroform layer indicated the presence of steroids.

Liebermann-Burchard test

1 ml of concentrated sulphuric acid was added to 10 mg of extract in 1 ml of chloroform. A reddish-blue color exhibited by the chloroform layer and green fluorescence by the acid layer suggests the presence of steroids.

Saponin test

1 ml solution of extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 min. Development of stable foam suggests the presence of saponins.

Tannin test

The test extract was taken in water, warmed and filtered. 5 ml of filtrate was allowed to react with 1 ml of 5% FeCl₃ solution. If dark green or deep blue color is obtained, it indicates the presence of tannins.

Formulation and characterization of hair tonic

The formula used in making this hair tonic preparation uses a modified hair tonic formula of 30, 35, and 40% of extract. Then as a comparison, a negative (blank) and a positive control (minoxidil 2%) were tested against the rats. The hair tonic preparation formula is in the form of a solution. The process of making hair tonic begins with weighing tween 80, dissolving it with distilled water, stirring until dissolved (mixture 1), weighing the thick extract of Broccoli stalks (*Brassica oleracea* var. *Italica*) then dissolving it with mixture 1 until completely dissolved (mixture 2). Weighed the sodium metabisulfite, dissolved it in distilled water until it dissolves, then mix it into mixture no. 2 and stir until homogeneous (mixture 3). Weighed methyl paraben and menthol, dissolved each in ethanol until dissolved, then mixed the two, stirred homogeneously (mixture 4). Weighed propylene glycol, added propylene glycol little by little into mixture no. 4, stirred until homogeneous. Incorporate mixture no. 3 into mixture no. 4 gradually, stirring until a uniform consistency is achieved. Next, scent was added and adjusted the volume by adding distilled water [15, 35, 39, 40].

Table 1: Formula modification of hair tonic preparation for broccoli stalks (*Brassica oleracea* var. *Italica*)

No	Ingredients	F0 (0%)	F1 (%)	F2 (%)	F3 (%)	Function
1.	Broccoli stalks extracts (%)	-	30	35	40	Active Ingredient
2.	Ethanol 70% (ml)	30	30	30	30	Solvent
3.	Na. Metabisulfite (g)	0.01	0.01	0.01	0.01	Preservative
4.	Methyl paraben (g)	0.25	0.25	0.25	0.25	Preservative
5.	Menthol (g)	0.1	0.1	0.1	0.1	Vasodilator
6.	Tween 80 (ml)	2	2	2	2	Emulsifier
7.	Propylene glycol (g)	15	15	15	15	Humectant
8.	Parfume (ml)	0.05	0.05	0.05	0.05	Fragrance
9.	Aquadest (ml)	52.59	22.59	17.59	12.59	Solvent

Physical characteristics of *Brassica oleracea* var. *Italica* hair tonic

Conducting tests on the physical qualities and characteristics of hair tonic formulations is a crucial step to perform once the formulation is prepared. The physical properties include the pH tests, homogeneity assessment, organoleptic evaluations and stability measurements [35, 41, 42].

Irritation test

A skin irritation test is conducted to observe the occurrence of erythema and edema by applying 2 drops of hair tonic into the skin area and then observed for 24 h. The observation of the irritation test includes the redness of skin, erythema and edema [43, 44]. The

animal has received approval from ethical clearance with number: 0697/KEPH-FMIPA/2021 Animal Research Ethics Committees (AREC) by University of North Sumatra, Indonesia.

Stability test

Physical stability tests were conducted by subjecting hair tonic preparations to storage temperatures of 4 °C and 40 °C using a cycle test. The samples were initially stored at a temperature of 4 °C for a duration of 24 h. Preparations were then transferred to a drying cabinet set at a temperature of 40 °C for another 24 h. This process, known as one cycle, lasted a total of 48 h. A total of 6 cycles were conducted over a span of 12 d. Each cycle involved evaluating the preparation through organoleptic observation, pH test, and the homogeneity of the preparations [45].

Preparation of animal testing

The study utilized 15 male white rats as tested animals. Rats were separated into 5 groups with a total 3 rats assigned to each group. Rats used in this study were *Rattus norvegicus domestica* aged above 2 months old, weighed about 450 g each. The rats were acclimatized for 3 d (72 h) prior for use in experiment. A 1×1 cm patch in the middle of the rat's back is shaved to measure the weight and length of its hair. A treatment box was constructed utilizing a marker. Depilatory lotion was applied to permanently remove hair from each rat. The cream was left on for 24 h. Afterward, hair tonic preparation was applied once daily to the rear of the male white rats for 21 d [20].

For the complete group of animal testing please see table 2.



Fig. 1: (A) the rat's hair is shaved to a length of 1×1 cm. (B) Rat is treated with hair tonic

Table 2: Treatment group for rat hair growth activity test

Formulation	Animal tested	Treatment
F0 (Negative control)	3	A blank was dropped that did not contain Broccoli stalks (<i>Brassica oleracea</i> var. Italica) extract.
F1	3	A hair tonic preparation containing 30% Broccoli stalks (<i>Brassica oleracea</i> var. Italica) extract was applied.
F2	3	A hair tonic preparation containing 35% Broccoli stalks (<i>Brassica oleracea</i> var. Italica) extract was applied.
F3 Positive control	3	A hair tonic preparation containing 40% Broccoli stalks (<i>Brassica oleracea</i> var. Italica) extract was applied.
	3	2% of minoxidil

Hair growth activity test of male white rat

The animal has received approval from ethical clearance with number: 0697/KEPH-FMIPA/2021 Animal Research Ethics Committees (AREC) by University of North Sumatera. The test formulation was administered topically on the rat's dorsal region, with a dosage of up to 1 ml each day for a duration of 21 d. For the negative control, hair tonic without Broccoli stalks (*Brassica oleracea* var. Italica) extract was applied to the backs of rats labelled 1, 2, and 3. Formula 1 consisted of a hair tonic comprising 30% extract of Broccoli stalks (*Brassica oleracea* var. Italica), which was applied to the backs of rats numbered 4, 5, and 6. A hair tonic mixture containing 35% extract of Broccoli stalks (*Brassica oleracea* var. Italica) was applied to the backs of rats numbered 7, 8, and 9. A hair tonic formulation, known as Formula 3 containing 40% extract of Broccoli stalks (*Brassica oleracea* var. Italica). Was applied to the backs of rats numbered 10, 11, and 12. Minoxidil, a

hair tonic, was applied as a positive control on the backs of rats numbered 13, 14, and 15 [20, 46].

RESULTS AND DISCUSSION

Phytochemical screening results

The ultimate yield obtained from 20 kg of fresh Broccoli stalks (*Brassica oleracea* var. Italica) raw material was 3000 gram of dry weight, resulting in a yield of 15%. The maceration method was employed to extract compounds from 3 kg of Broccoli heads (*Brassica oleracea* var. Italica) using 30 liters of 70% ethanol solvent. The resulting extract was concentrated using a rotary evaporator and the thick extract obtained was 350 g with a yield of 11.6%. Fresh Broccoli, dried Broccoli stalks and extract of Broccoli stalks can be seen in fig. 2. Table 3 displays the data from the phytochemical screening test conducted on the extract of Broccoli (*Brassica oleracea* var. Italica). The phytochemical screening test for Broccoli (*Brassica oleracea* var. Italica) extract indicates the presence of flavonoids, alkaloids, terpenoids, steroids and saponins.

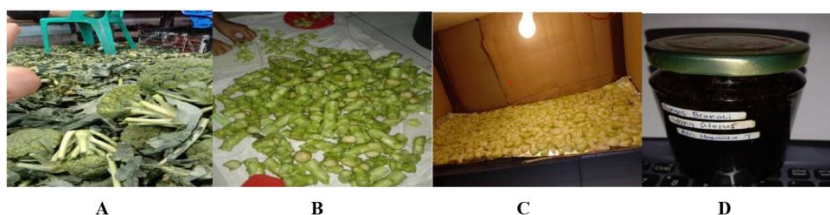


Fig. 2: (A) Fresh broccoli, (B) Fresh broccoli stalks. (C) Dried broccoli stalks, (D) Extract

Table 3: Phytochemical screening results of *Brassica oleracea* var. *italica*

Screening test	Reagent test	Result
Alkaloid	Dragendorff	+
	Maeyer	+
Flavonoid	Mg. HCL	-
Terpenoid	H ₂ SO ₄	+
Steroid	Liberman-Burchard	-
	Salkowaski	+
Saponin	Aquadest	+
Tanin	FeCl ₃	-

NB:+: Positive.-: Negative

Organoleptic inspection

Organoleptic observations of the three hair tonic formulas showed that the resulting preparations were not transparent. This is due to the use of *Brassica oleracea* var. *italica*, which is a thick extract so that the resulting color is thick. Organoleptic examination serves to

determine the appearance of hair tonic, color and aroma, which is carried out visually during 6 storage cycles, namely for 12 d. Organoleptic testing was carried out in each cycle, where 1 cycle consisted of storage at a temperature of 4 °C for 24 h and a temperature of 40 °C for 24 h. The results of the hair tonic organoleptic test can be seen in table 4 and fig. 3.

Table 4: Organoleptic test of *Brassica oleracea* var. *italica* hair tonic

Formulation	Test	Cycle							
		0	1	2	3	4	5	6	
F0	Shape	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
	Smell	Distinctive	Distinctive	Distinctive	Distinctive	Distinctive	Distinctive	Distinctive	Distinctive
	Color	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear
F1	Shape	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
	Smell	Distinctive	Distinctive	Distinctive	Distinctive	Distinctive	Distinctive	Distinctive	Distinctive
	Color	Light brown	Light brown	Light brown	Light brown	Light brown	Light brown	Light brown	Light brown
F2	Shape	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
	Smell	Distinctive	Distinctive	Distinctive	Distinctive	Distinctive	Distinctive	Distinctive	Distinctive
	Color	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown
F3	Shape	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
	Smell	Distinctive	Distinctive	Distinctive	Distinctive	Distinctive	Distinctive	Distinctive	Distinctive
	Color	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown



Fig. 3: Hair tonic of 30%, 35%, 40% and 0% of *Brassica oleracea* var. *italica* extract

The organoleptic examination results indicate that the hair tonic preparation in F0 (0% extract) is in a liquid form with a transparent color and a noticeable menthol fragrance. F1 hair tonic is created by incorporating a 30% concentration of *Brassica oleracea* var. *italica* stalks extract, this tonic is in liquid form, has a light brown hue and possesses a unique aroma. By incorporating 35% extract of the *Brassica oleracea* var. *italica* stalks (F2) showed tonic is in liquid form, has a dark brown color, and possesses a unique aroma. F3 is made by using 40% extract from the Broccoli stalks (*Brassica oleracea* var. *italica*) resulting in a liquid hair tonic with dark brown color and is characterized by a unique aroma. From 0 to 6 cycles of

stability tests (12 d), there were no discernible alterations in terms of viscosity, color and fragrance [20, 47, 48].

The organoleptic test is a visual examination that encompasses the assessment of form, odor, and color. The experiment was conducted utilizing the cycling test methodology. Specifically involving 6 cycles. Organoleptic observations were conducted from the initial cycle to the sixth cycle. Organoleptic analysis was conducted on hair tonic formulations to assess the stability of the product in terms of its physical appearance, color, and fragrance. The organoleptic test indicated that the preparation remained unchanged in terms of form, aroma, and color. While being subjected to different temperatures. Specifically 4 °C and 40 °C. The hair tonic preparation. Broccoli stalks extract (*Brassica oleracea* var. *italica*). Passes the hair tonic quality test based on the organoleptic evaluation [48].

The organoleptic test is a visual examination that encompasses the characteristics of shape. Fragrance and color. The test was conducted utilizing the cycling test method, namely with 6 cycles. Organoleptic observations were conducted from the initial cycle to the sixth cycle. The hair tonic formulation without extract (F0) appears transparent upon observation. The 30% concentration exhibits a light brown color, while the 35% and 40% concentrations showed a dark brown hue. Among the four formulations. There is no indication of a change in color from cycle 0 to cycle 6. The color in the hair tonic mixture at concentrations of 30%, 35%, and 40% is derived from the addition of stalks extracted from the *Brassica oleracea* var. *italica*. The form observed at concentrations of 30%, 35%, and 40% exhibits a liquid form. The formulation of hair tonic remains stable from cycle 0 to cycle 6 during the stability tests. The odor of the hair tonic derived from the fragrance added in formula and did not change in cycles 0 to 6 during the stability test.

pH test

The pH test results for the hair tonic preparation of Broccoli stalks extract (*Brassica oleracea* var. Italica) are displayed in fig. 4. The test findings indicated that F0, F1, F2, and F3 exhibited pH values within the range of 5-6.5. The test results indicate that hair tonic in F0, F1, F2, and F3 meet the quality criteria for skin preparation. Namely within the pH range of 4-7 [20, 47].

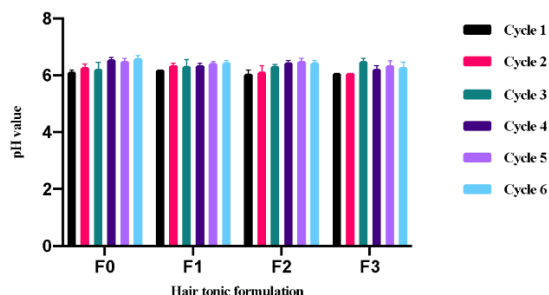


Fig. 4: pH test result of *Brassica oleracea* var. Italica hair tonic (n=3). Error bars indicate the SD values.

Conducting a pH test on the hair tonic formulation, the extract of the broccoli stalks (*Brassica oleracea* var. Italica) was produced at a pH level ranging from 5 to 6.5. The purpose of the pH test is to determine the level of acidity in the hair tonic preparation, ensuring its safety and non-irritating properties for the skin. The hair tonic preparation was subjected to a cycle test method for 12 d, with temperatures maintained at 4 °C and 40 °C. The pH of the

preparation remained constant during the test period. The pH test findings indicate that all formulations meet the hair tonic quality standards. As they have a pH within the range of 4-7. Extreme pH levels, whether very high or very low, can enhance the skin's ability to absorb substances, thereby leading to skin irritation. The hair tonic preparation. Formulated with Broccoli (*Brassica oleracea* var. Italica), stalks extract (*Brassica oleracea* var. Italica) has a pH value ranging from 5 to 6.5. This pH range ensures that the hair tonic preparation meets the necessary parameters (fig. 4).

Homogeneity test

The homogeneity test is conducted by applying the preparation onto a glass plate and observing for any presence of non-uniformly dispersed particles. The homogeneity observations yielded data which are presented in table 5. This examination was conducted by observing the formulation of the hair tonic. The hair tonic is considered to meet the homogeneity criteria when it does not contain any particles. Uncombined components or clumps in its formulation or stable after 6 cycles [49].

The homogeneity test findings of hair tonic preparations obtained at F0, F1, F2, and F3 indicate that the preparations are homogeneous [45]. This is evidenced by the absence of lumpy sections, equal color distribution, and the absence of lumpy particles. Based on the results of the homogeneity test, it was determined that the hair tonic preparation fulfilled the criteria.

Irritation test

Data on the results of the irritation test for the Hair Tonic preparation Broccoli stalks extract (*Brassica oleracea* var. Italica) can be seen in table 6. Based on the results of the irritation test carried out, it shows that F0, F1, F2, F3 do not cause skin irritation such as redness, erythema and edema.

Table 5: Homogeneity test results for hair tonic preparation of broccoli stalk extracts

Formula	Test	Cycles						
		0	1	2	3	4	5	6
F0	Homogeneity	S	S	S	S	S	S	S
F1	Homogeneity	S	S	S	S	S	S	S
F2	Homogeneity	S	S	S	S	S	S	S
F3	Homogeneity	S	S	S	S	S	S	S

Note: S: Stable; NS: Not Stable

Table 6: Irritation test

Formula	Observation	Animals		
		I	II	III
F0	A	-	-	-
	B	-	-	-
	C	-	-	-
F1	A	-	-	-
	B	-	-	-
	C	-	-	-
F2	A	-	-	-
	B	-	-	-
	C	-	-	-
F3	A	-	-	-
	B	-	-	-
	C	-	-	-

A: Redness. B: Erythema C: Edema

Hair tonic is a recurring preparation that is applied daily; hence its safety, efficacy, and the usage of hair tonic are compromised when it induces adverse skin reactions such as redness, erythema, and edema. Irritation test is needed to ascertain the presence or absence of a skin reaction in the form of irritation. Observation of irritation entails the examination of alterations in the skin such as redness, erythema, and edema subsequent to the application of hair tonic. According to the results of the irritation test (table 6), it was determined that the

application of preparations F0, F1, F2, and F3 did not cause any adverse effects such as redness, erythema, and edema on the skin. The safety of Broccoli stalks extract (*Brassica oleracea* var. Italica) hair tonic preparation has been confirmed by the irritation test.

Test the activity of hair tonic preparations on hair growth

Data on the average growth of rat hair length per treatment per week can be seen in fig. 5 below. The hair growth activity test is

assessed using the mean hair growth test outcomes [50]. This experiment aimed to assess the impact of different concentrations of Broccoli stalks (*Brassica oleracea* var. Italica) extract on the rate of hair growth in rats. Measurements of hair length in rat were conducted on the 7th, 14th, and 21st d. The rat's hair was measured using a digital caliper [20, 32]. The hair growth measurements were subsequently subjected to statistical analysis using the ANOVA test to determine if there were any significant variations between the test region and the positive

control. The One-Way ANOVA test results indicate a P value of <0.05 (P=0.000). Indicating a significant difference between the treatment groups. In various previous studies, it has been established that the utilization of alkaloid compounds present in the broccoli plant has demonstrated a positive impact and response in the process of hair growth. Therefore, as the percentage of broccoli extract in the hair tonic increases, there is an improvement in the hair growth rate observed from the 7th w, 14th w, up to the 21st w [21, 34, 51].

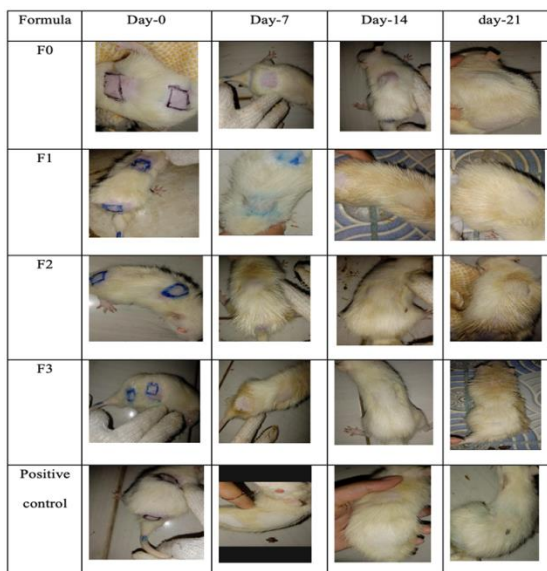


Fig. 5: Result of hair tonic formulation on rats hair growth in day 0, day 7, day 14, and day 21

ANOVA test results on day 21

The results of data processing show that the control (+) has a significant difference in hair growth compared to the control (-), F1, F2 where the significance value is <0.05, but does not show a significant difference to F3. F1 shows a significant difference to the other four formulas where the significance value is <0.05. F2 has a significant difference to positive control (+). Negative control (-) and F1, but does not show a significant difference to F3. In F3 there is a significant difference to F1 and control (-) but there is no significant difference to F2 and control (+) with a significance value of >0.05. This shows that Broccoli stalks extract (*Brassica oleracea* var. Italica) has activity on growth hair was not much different from the positive control and it could be concluded that Formula 3 (40%) was the most effective in rat hair growth (fig. 6).

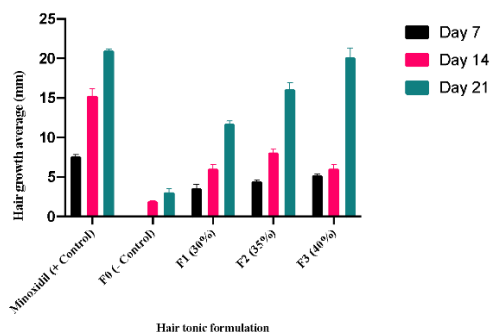


Fig. 6: Result of hair tonic formulation on representation of statistical fig. after day 7, day 14, and day 21 (n=3)

CONCLUSION

The utilization of broccoli stalks extract (*Brassica oleracea* var. Italica) enables the production of a hair tonic formulation that

maintains its stability over time and successfully meets the physical criteria for hair tonic preparations. The extract obtained from the *Brassica oleracea* var. Italica, commonly referred to as broccoli stalks, has the capacity to promote hair development. The most effective formula for stimulating rat hair growth is the extract obtained from 40% of the stalks from *Brassica oleracea* var. Italica.

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AUTHORS CONTRIBUTIONS

Benni Iskandar: Conceptualization, methodology, Investigation, data curation, writing. Pricella Aqwilla Ginting: Writing-review and editing, funding. Endang S. Sitorus: Methodology, investigation, data curation. Writing review and editing. Akhmad Fajri Widodo: Writing-review and editing, data curation. Wei-Ching Peng: writing-review and editing, data curation. Ieny: Conceptualization, supervision, writing-review and editing, acquisition.

CONFLICT OF INTERESTS

The authors have no conflict of interest associated with the material presented in this paper.

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