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FORMULATION OF HAIR TONIC COMBINATION OF ETHANOL EXTRACTS OF *MORINGA* OLEIFERA LAM LEAVES AND FRAGRANT PANDAN (*Pandanus Amaryllifolius Roxb*) AND ITS ACTIVITY ON HAIR GROWTH IN WHITE RATS

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ABSTRACT

Hair is the crown for everyone because hair functions aside from providing warmth, protection, beauty, and support. Hair loss that can cause baldness is one of the most serious problems for everyone. This study aimed to determine the activity of *Hair Tonic* combination formulations of kelor leaf ethanol extract (*Moringa oleifera Lam.*) and ethanol extracts of *Fragrant Pandan* leaves (*Pandanus amaryllifolius Roxb*) on rat hair growth compared to single use. The method began with the maseration process of ethanol extract of kelor leaves and ethanol extract of Fragrant Pandan leaves, characterization of extracts (specific and non-specific parameters), chemical content testing of extracts and formulation of Hair Tonic preparations consisting of F1 (positive control), F2 (6% kelor leaf extract), F3 (6% ethanol pandan extract), F4 (combination of 2% kelor leaf extract with 4% ethanol pandan extract), F5 (3% kelor leaf ethanol extract and 3% ethanol pandan leaf extract), F6 (combination of 4% kelor leaf ethanol extract and 2% ethanol pandan leaf extract). The rat hair growth activity was divided into 8 groups: each rat's back was shaved to a size of 4 × 4 cm, the rats were left for 24 hours and then testing was carried out by spraying each formula, and hair growth was observed for 28 days. The results of the research were the organoleptic properties of kelor leaf and pandan leaf ethanol extract Hair Tonic formulations in the form of liquid with a characteristic odor, green to blackish green color, and blackish brown, with pH values ranging from 4.5 ± 0.00 - 5.8 ± 0.01. The viscosity values ranged from 1.64 ± 0.00 - 1.82 ± 0.01 cPs. The specific gravity of the formulations was 0.97 ± 0.00-0.99 ± 0.00 g/mL. The rat hair length results for 30 days with kelor leaf and pandan leaf extract concentrations ranged from 2.296 ± 0.15 - 7.532 ± 0.08 mm. The weight of hair in rats ranged from 0.644 ± 0.03-0.904 ± 0.04 g. Based on the results of this study, it was concluded that the variation in the combinations of kelor leaf and pandan leaf extracts did not cause a significant difference in rat hair growth activity. The length of rat hair from the Hair Tonic combination formulation treatment was larger than that from the single treatment, but not significantly different. The activity of the combination Hair Tonic was significantly different (higher) in terms of rat hair weight compared to the single extract Hair Tonic.

Keywords: *Moringa Leaves*, *Fragrant Pandan Leaves*, *Hair Tonic*, Preparation Evaluation, Hair Length, Hair Weight

INTRODUCTION

Hair is often referred to as the crown for everyone, as it serves not only functional purposes, such as providing warmth and protection, but also plays a significant role in beauty

and overall appearance. Hair is found throughout the body and serves various functions, including human aesthetics. It is often considered a symbol of femininity for women, whereas for men, hair can greatly influence self-confidence. Hair loss leading to baldness is a common concern for people of all genders (Sari & Wibowo, 2016). In the United States, hair shedding affects approximately 50 million people, of whom 20 million are women (Sugirno, 2021). In Indonesia, the prevalence of hair shedding is reported to be 39.7%, followed by Telogen Effluvium (TE) at 34.5%, and alopecia areata AA at 11.2% (Legiawati *et al., 2022)*.

Hair loss is a disorder or abnormality in which hair is released from the scalp or body skin, affecting various biological functions of hair in the body. Effective treatment can help prevent further hair loss and stimulate hair growth (Lensing & Jabbari, 2022). On average, individuals naturally lose 50-100 hairs per day through shedding, but most of these shed hairs regrow with the use of a Hair Tonic. Hair Tonics offers advantages such as ease of use, suitability for the scalp, ease of spreadability, non-oiliness, and the absence of residues that may cause scalp irritation (Mustarichie *et al*., 2019). A hair Tonic is a medication used to strengthen hair roots, stimulate hair growth, cleanse the scalp, and provide hair moisturization. Hair growth stimulants (Hair Tonic) are formulated with ingredients that are beneficial to hair, hair roots, and scalp (Sanjiwani *et al*., 2020). The mechanism of action of the Hair Tonic involves stimulating the growth of hair follicles that contain melanocyte cells responsible for producing melanin (hair color or pigment) and cells that synthesize hard keratin, which forms the foundation for healthy, shiny, and resilient hair. Hair Tonics are commonly formulated using plant extracts (Fang *et al*., 2023).

Moringa leaf extract, known for its rich nutrient content, is widely recognized as a natural ingredient that promotes scalp and hair health. It contains secondary metabolites such as saponins, alkaloids, quinones, and flavonoids, which have been found to stimulate hair growth (Syahputra *et al*., 2016). Research suggests that 1% concentration of *Moringa* leaf extract is optimal for stimulating hair growth. Compared to the positive control minoxidil 2.5%, the 1% *moringa* leaf extract resulted in an average hair length of 3 cm and hair weight of 0.236 g, whereas minoxidil resulted in an average hair length of 2.025 cm and hair weight of 0.207 g (Feraldy *et al*., 2023). Minoxidil stimulates hair growth in the absence of a blood supply in hair follicle cell cultures and acts as an enzymatic cytoprotective activator of prostaglandin endoperoxide synthase-1, promoting hair growth (Herman, 2016). Fragrant Pandan leaves (*Pandanus amaryllifolius Roxb*), commonly used as flavor enhancers in food, are easily accessible natural ingredients. They contain secondary metabolites such as alkaloids, saponins, flavonoids, tannins, polyphenols, and colorants (Wilujeng *et al*. 2020). The alkaloids, saponins, and polyphenols present in the *Fragrant Pandan* leaves contribute to hair health. Flavonoids accelerate hair growth and prevent hair loss, whereas saponins increase blood flow to the hair follicles (Feraldy *et al*., 2023). The 5% Fragrant Pandan leaf extract exhibited the best hair growth activity. When formulated as a Hair Tonic, it effectively stimulated hair growth, resulting in an average hair length of 12.02 mm and a hair weight of 90.95 mg on day 28 (Septiani *et al*., 2021).

Recently, there has been a shift in treatment approaches from conventional drugs that rely on single chemical compounds targeting a specific goal (one drug target) to herbal-based multicomponent treatments involving several chemical compounds that work on one or more targets (multicomponent-network target). Interactions between combinations of active ingredients in multicomponent drugs can lead to synergistic or antagonistic effects. The aim of developing herbal medicines is to achieve synergistic effects between the active ingredients. Synergistic combinations are highly sought after in the current development of herbal medicines (Hilal A. Syahrir *et al*., 2016).

RESEARCH METHODS

This study employed experimental research conducted in a laboratory using a post-test control group design to evaluate the efficacy of hair growth when administering a Hair Tonic containing *moringa* leaf extract and fragrant Pandanus extract.

Equipment and Materials

The tools used in this study included parchment paper, glassware (Pyrex), spatula, stir rod, funnel, dropper, tissue, filter paper, plastic bottles, digital pH meter, Ostwald viscometer (IKA®), rotary evaporator (IKA®), hair clipper, marker pen, oven (Memmert®), and furnace (Thermolyne®). The materials employed comprised *Moringa* leaves (*Moringa oleifera* Lam) and fragrant Pandan leaves (*Pandanus amarillyfolius* Roxb) obtained from Pereng Wetan Village, Prambanan Subdistrict. Concentrated HCl, Mayer's reagent, 1 N HCl, acetic acid anhydride, chloroform, FeCl3, 96% ethanol, propylene glycol, methylparaben, propylparaben, sodium metabisulfite, menthol, and distilled water were also used.

Research Procedure

1. **Chemical Content Testing of Extracts:**
   a. **Saponin Identification**

   Approximately 0.5 g of the extract was placed in a reaction tube. Subsequently, 10 mL of hot water was added, followed by cooling and vigorous shaking for 30 seconds. The formation of stable foam, measuring 1 to 10 cm in height that lasted for at least 10 minutes indicated the presence of saponins (Hasan Khan *et al*., 2019).

   b. **Flavonoid Identification**

   For flavonoid identification, the extract (0.5 g) was mixed with 5 mL distilled water. The mixture was then boiled for 5 minutes and filtered. To the filtrate, 1 mL concentrated HCl and a small amount of Mg powder were added. Shaking the mixture would result in red, yellow, or orange color if flavonoids were present (Hasan Khan *et al*., 2019).

   c. **Polyphenol Identification**

   0.5 grams of the extract was mixed with 5 mL distilled water. The mixture was boiled for 5 minutes and filtered to obtain filtrate. Then, five drops of 1% FeCl3 solution were added to the filtrate, and any color change was observed. The presence of polyphenol compounds was indicated by a color change from blue to green to black (Hasan Khan *et al*., 2019).

   d. **Alkaloid Identification**

   0.5 grams of extract was mixed with 4-5 drops of Mayer's reagent. The formation of a white precipitate indicates the presence of alkaloids in the sample (Hasan Khan *et al*., 2019).

   e. **Tannin Identification**

   Approximately 0.5 g of the extract was diluted with water and heated in a water bath. FeCl3 reagent was then added, and the formation of a green color indicated the presence of tannins. Additionally, when gelatin was added, a white precipitate formed, further confirming the presence of tannins (Hasan Khan *et al*., 2019).

2. **Hair Tonic Preparation Formulation**

The Hair Tonic preparations were formulated using single extracts of *moringa* leaves, pandan leaves, and combinations of *moringa* leaf extract and pandan leaf extract. Six different concentrations of *Moringa* leaf extract combined with pandan leaf extract were utilized. The details of each Hair Tonic formulation can be found in **Table I**. The Hair Tonic preparation formula is also outlined in **Table I.**

Table I. Formulation of Hair Tonic Preparations Combining *Moringa* Leaf Ethanol Extract and Fragrant Pandan Leaf Ethanol Extract.

Material	The Formula (%)							
	F1	F2	F3	F4	F5	F6	F7	F8
Moringa leaf extract	-	6	-	2	3	4	-	-
fragrant Pandan leaves extract	-	-	6	4	3	2	-	-
Propylene glycol	15	15	15	15	15	15		
Methyl paraben	0.3	0.3	0.3	0.3	0.3	0.3	-	-
96% ethanol	35	35	35	35	35	35	-	-
Sodiummeta bisulphate	0.03	0.03	0.03	0.03	0.03	0.03	-	-
Menthol	0.3	0.3	0.3	0.3	0.3	0.3	-	-
Aquadest ad	100	100	100	100	100	100	-	100

Notes :
Formula 1 served as the negative control (-) and did not contain *Moringa* leaf extract and pandan leaf extract (F1).
Formula 2 contained 6% *Moringa* leaf extract (F2).
Formula 3 contained 6% pandan leaf extract (F3).
Formula 4 comprised a combination of 2% *Moringa* leaf extract and 4% pandan leaf extract (F4).
Formula 5 consisted of a combination of 3% *Moringa* leaf extract and 3% pandan leaf extract (F5).
Formula 6 included a combination of 4% *Moringa* leaf extract and 2% pandan leaf extract (F6).
Formula 7 A positive control (+), consisting of Aloe vera Natur Hair Tonic (F7), was used.
Formula 8 Normal group (F8)

3. Formulation Evaluation

a. Organoleptic Test

The *Hair Tonic* preparations were visually inspected for changes in color, aroma, odor, and overall appearance. Organoleptic testing was performed on days 0, 7, 14, 21, and 28 to assess the stability and sensory characteristics of the preparations (Lailiyah, 2023).

b. pH Test

The pH of the *Hair Tonic* preparations was measured using a digital pH meter. The instrument was calibrated using standard solutions of pH 4 and pH 7. The pH meter electrode was immersed in the preparation and the pH value was recorded. Measurements were conducted at room temperature on days 0, 7, 14, 21, and 28 (Dwivedi *et al*., 2023).

c. Viscosity Test

The viscosity measurements were performed using an Ostwald viscometer. A volume of 10 mL of *Hair Tonic* was introduced into the viscometer tube, and the Hair Tonic was allowed to flow from the upper limit to the lower limit. The time required for the Hair Tonic to flow was recorded using a stopwatch. The viscosity test was repeated three times, with measurements taken every week for a period of one month. Viscosity testing was performed on days 0, 7, 14, 21, and 28 (Sanjiwani *et al*., 2020) .Viscosity values were calculated using Equation 1:

$$\eta \text{ sample} = \frac{\eta \text{ water} \times \rho \text{ sample} \times t \text{ sample}}{\rho \text{ water} \times t \text{ water}} \times 100\% \quad \text{.....(1)}$$

Notes: η1 = viscosity of water (0.89 cps) (Water has a viscosity of 0.00899 poise at 25 °C and 1 atm (0.00899 P = 0.899 cP = 0.899 mPa·s))
η2 = viscosity of Hair Tonic (sample) (cps)
ρ1 = density of water (g/mL)
ρ2 = density of Hair Tonic (sample) (g/mL)
t1 = time of water (seconds)
t2 = time of Hair Tonic (sample) (seconds) (Sanjiwani *et al*., 2020)

d. Density Testing

The density of the *Hair Tonic* preparations was determined by using a clean and dry pycnometer. An empty pycnometer (W1) was weighed at room temperature. It was then filled with distilled water, and the outer part of the pycnometer was dried and weighed (W2). Distilled water was poured out and the pycnometer was dried again. Next, the hair was filled with the Hair Tonic preparation to be measured for density and weighed (W3). Density testing was conducted on days 0, 7, 14, 21, and 28 to assess changes over time. The density of the *Hair Tonic* was calculated using equation 2 (Heroweti *et al*., 2023).

$$\text{Density Testing} = \frac{W3-W1}{W2-W1}\ x\ 100\% \quad \text{.....(2)}$$

Notes : W1= Empty pycnometer weight, W2 = Weight of the pycnometer filled with distilled water, W3= Weight of the pycnometer filled with Hair Tonic preparation

4. Rat Hair Growth Test

Prior to the test, the rats were acclimatized for 1 week and then divided. The hair on the back of each rat was shaved using a 4x4 cm hair clipper. After 24 hours, the testing was initiated. The areas for spraying were determined evenly to account for the possibility of different hair growths in each area. By ensuring even spraying, it was anticipated that the hair growth activity in all areas could be represented. The formulations were sprayed twice a day (morning and afternoon) at a volume of 1 mL per formulation. The spraying was done from a distance of 10 cm from the test animal. This test aimed to assess the efficacy of the Hair Tonic formulations when sprayed and evaluate the quality of the spray pattern produced. A good Hair Tonic spray formulation can be easily sprayed from the applicator with light pressure and forms a well-spread spraying pattern.
F1 : Rats given a formulation without *Moringa* leaf extract and pandan wangi extract (negative control)
F2: Rats administered a formulation containing 6% *moringa* leaf extract (F2).
F3: Rats given a formulation containing 6% Fragrant Pandan leaf extract (F3).
F4: Rats administered a combination of 2% *Moringa* leaf extract and 4% *Fragrant Pandan* leaf extract (F4).
F5: Rats were given a combination of 3% *moringa* leaf extract and 3% *Fragrant Pandan* leaf extract (F5).
F6: Rats were given a combination of 4% *moringa* leaf extract and 2% *Fragrant Pandan* leaf extract (F6).
F7: Rats administered the natural *hair tonic aloe vera* formula as a positive control (F7).
F8: Rats were not administered any treatments (F8).

Observations were conducted over a period of 29 days. The spraying areas were evenly distributed to ensure representative hair growth activity in all regions. Hair plucking was performed using tweezers to facilitate observation. Among the 20 plucked hairs, the 10 longest hairs were selected and measured. Hair plucking was performed once a week. The shaved hair was weighed and the hair length was measured. These results were statistically analyzed to assess hair growth and development on day 30 (Rahmi *et al*., 2021).

Data Analysis

The data obtained in the study were statistically processed using IBM SPSS Statistics software (version 25.0), starting with a normality test using *the Kolmogorov-Smirnov* test and a homogeneity test using *Levene*'s test. The data obtained were normal and homogeneous so that they were continued using the two-way ANOVA test with independent factors (formula and time) and dependent factors (hair length) and continued with the *Tukey test*. The hair weight data were analyzed using the one-way ANOVA test and continued with the *Tukey test*.

RESULTS AND DISCUSSION

Phytochemical Screening Of *Moringa* Leaf And Fragrant Pandan Leaf Ethanol Extracts Additional phytochemical tests were conducted on the ethanol extracts of *moringa* leaf and Fragrant Pandan leaf to determine the presence of various compounds, including saponins, flavonoids, polyphenols, alkaloids, and tannins. The results revealed that both *Moringa* leaf and Fragrant Pandan leaf extracts tested positive for saponin compounds, as indicated by the formation of foam. Flavonoids were detected by the development of a yellow color, whereas polyphenols were detected by a green-black color. The presence of alkaloid compounds was confirmed by the formation of a white precipitate, and a green solution identified tannin compounds. These findings are consistent with the study conducted by (Syahputra *et al*., 2016). Detailed information regarding the identified chemical compounds is provided in **Table II**.

Table II. Results of Phytochemical Screening of *Moringa* Leaf Extract Combined with Fragrant Pandan Leaf Extract

Phytochemical Testing	*Moringa* Leaf	Pandan Leaf
Saponin	+	+
Flavonoid	+	+
Polyphenol	+	+
Alkaloid	+	+
Tannin	+	+

Note : - (+) = indicating the presence of compounds and - (-) = indicating the absence of compound

Organoleptic tests were conducted to assess the potential physical instability of the Hair Tonic preparation, specifically focusing on the color, aroma, and dosage form throughout the storage period. The observations revealed that formulations F1, F2, F3, F4, F5, F6, and F7 did not exhibit any noticeable changes in color, aroma, and shape from week 0 to week 4, and the Hair Tonic preparations yielded distinct characteristics based on each formulation. F1 resulted in a clear-colored preparation with a distinct menthol aroma that maintained a liquid form. F2 exhibited a brownish-black color, distinct menthol aroma, and liquid form. F3 had a greenish-brown color, distinct menthol aroma, and maintained its liquid form. F4 exhibited a green color with a distinct menthol aroma in liquid form. F5, F6, and F7 produced a brown color, menthol aroma, and remained in liquid form.

Based on the organoleptic evaluation of the Hair Tonic preparation containing the ethanol extract of *moringa* leaves combined with that of fragrant pandan leaves, it can be concluded that the preparation remained stable without any changes during the storage period. This indicates that the preparation is of good quality, as it maintains its original characteristics (Budastra *et al*., 2023), as shown in **Table III**.

Table III. Organoleptic Results of The Hair Tonic Formulation From A Combination of Ethanol Extract Of *Moringa* Leaves and Ethanol Extract of Fragrant Pandan Leaves

Parameter	Formula	Length of Observation				
		Day-0	Day-7	Day-14	Day-21	Day-28
Colour	F1	Clear	Clear	Clear	Clear	Clear
	F2	Brownish Black	Brownish Black	Brownish Black	Brownish Black	Brownish Black
	F3	Black	Black	Black	Black	Black
	F4	Green	Green	Green	Green	Green
	F5	Brown	Brown	Brown	Brown	Brown
	F6	Brown	Brown	Brown	Brown	Brown
	F7	Brown	Brown	Brown	Brown	Brown
Smell	F1	Menthol	Menthol	Menthol	Menthol	Menthol
	F2	Menthol	Menthol	Menthol	Menthol	Menthol
	F3	Menthol	Menthol	Menthol	Menthol	Menthol
	F4	Menthol	Menthol	Menthol	Menthol	Menthol
	F5	Menthol	Menthol	Menthol	Menthol	Menthol
	F6	Menthol	Menthol	Menthol	Menthol	Menthol
	F7	*Aloe Vera*	*AloeVera*	*Aloe Vera*	*Aloe Vera*	*Aloe Vera*
Flavor	F1	Fluid	Fluid	Fluid	Fluid	Fluid
	F2	Fluid	Fluid	Fluid	Fluid	Fluid
	F3	Fluid	Fluid	Fluid	Fluid	Fluid
	F4	Fluid	Fluid	Fluid	Fluid	Fluid
	F5	Fluid	Fluid	Fluid	Fluid	Fluid
	F6	Fluid	Fluid	Fluid	Fluid	Fluid
	F7	Fluid	Fluid	Fluid	Fluid	Fluid

pH tests were conducted on formulations F1, F2, F3, F4, F5, F6, and F7 at various time points, from week 0 to week 4. The purpose of these tests was to determine whether the pH of the preparations was within the range suitable for the skin. The pH values were measured and recorded for each formulation. The results of the pH examination indicated that the overall pH values ranged from 4.5 ± 0.00 to 5.8 ± 0.01. According to the Indonesian National Standard (SNI) number 16-4955-1998, the recommended pH range for Hair Tonic preparations suitable for the scalp is 4.5-6.5. Based on the results of pH evaluation, it can be concluded that all formulated Hair Tonic preparations achieved an acceptable pH level. A previous study (Darajati & Ambari, 2021) supports this finding and indicates that the pH of the preparation gradually decreases over time or becomes more acidic. This change was attributed to the interactions between the ingredients in the preparation and the extreme temperature fluctuations experienced, ranging from cold to hot. The observed decrease in pH was primarily influenced by the entry of C_{O2} into the container during measurement. C_{O2} reacts with water, resulting in an acidic pH shift. These findings align with the study conducted by (Darajati & Ambari, 2021) highlighting the impact of C_{O2} on the pH of the Hair Tonic preparation. Overall, pH evaluation confirmed that the formulated Hair Tonic preparations maintained an acceptable pH level, despite the observed decrease over time due to the influence of C_{O2} (Darajati & Ambari, 2021). Next, a statistical analysis was conducted on the pH data using the one-sample Kolmogorov-Smirnov normality test. The analysis, carried out using the SPSS 25.0 program, revealed that the pH data for formulations F1-F7 followed a normal distribution, as indicated by a P-value of ≥0.05. Additionally, the homogeneity test, performed using the *Levene* Test, yielded a non-significant P-value ≥0.05, indicating that the data were homogeneous. These results confirm that the pH data satisfied the assumptions of normality and homogeneity, allowing for the continuation of the two-way comparison test, specifically the two-way ANOVA test. The subsequent analysis involved conducting a Between-Subjects Effects test to examine the effects of formula variations and

observation time (days). The obtained significance value of ≤0.05 indicated that both formula variations and observation time had a significant impact on the pH data. Consequently, further analysis using a *Post Hoc* Test is warranted. The Hoc Test serves as a post-hoc test to assess and compare significant differences between groups. are listed in **Table IV**.

Table IV. Hair Tonic pH Test Combination of *Moringa* Leaf Extract and Fragrant Pandan Leaf Extract.

Formula	pH Test				
	Day- 0	Day-7	Hari Ke 14[h]	Hari Ke 21[h]	Hari Ke 28[h]
F1	5.0±0.00 [bcdefg]	5.0±0.04	5.1±0.00[c]	5.4±0.00[c]	5.3±0.02[c]
F2	5.4±0.00 [adefg]	5.4±0.02	5.5±0.02	5.5±0.02	5,5±0.00
F3	5.6±0.02 [adefg]	5.6±0.00	5.7±0.00	5.8±0.00	5.8±0.01
F4	5.6±0.00 [abcg]	5.7±0.00	5.7±0.06	5.7±0.00	5.8±0.00
F5	5.6±0.02 [abcg]	5.7±0.00	5.7±0.00	5.7±0.00	5.8±0.00
F6	5.6±0.00 [abcg]	5.6±0.00	5.6±0.00	5.7±0.10	5.8±0.00
F7	4.5±0.00 [abcdef]	4.5±0.00	4.5±0.00	4.5±0.00	4.5±0.00

Notes:
[a] = there is a significant difference in pH with F1, [b]= there is a significant difference in pH with F2 [c]= there is a significant difference in pH with F3, [d]= there is a significant difference with F4 [e]= there is a significant difference with F5 [f]= there is a significant difference with F6 [g] = there is a significant difference with F7, [h] = there is a significant difference in pH with pH on day 0

The viscosity of a formulation plays a significant role in determining the consistency of a product when applied to the scalp. The viscosity calculations revealed that the overall viscosity values of the Hair Tonic formulations ranged from 1.64 ± 0.00 to 1.82 ± 0.01 cPs. The results indicated an increase in viscosity for each formulation, with higher concentrations of the extract resulting in thicker viscosities of the preparations. The observed viscosity values for the Hair Tonic formulations complied with the specified regulations, which state that the viscosity should be below 5 cPs (Darajati & Ambari, 2021). These findings highlight the need for careful control of extract concentration to achieve the desired viscosity levels in Hair Tonic preparations. By understanding and managing the viscosity of the formulations, it is possible to ensure the appropriate consistency of the Hair Tonic product, providing optimal user experience and efficacy (Budastra *et al*., 2023). Based on the results of viscosity evaluation, it can be inferred that all formulated Hair Tonic preparations possess acceptable viscosity levels. In line with this, research conducted by (Putri *et al*., 2023) supports the notion that the Hair Tonic preparation, comprising a combination of pandan leaf extract and gotu kola herb, underwent changes following its formulation. Each formulation exhibited an increase in viscosity, which could be attributed to solvent evaporation during the storage period (Putri *et al*., 2023). Subsequently, statistical analysis and viscosity differences were examined using the one-sample Kolmogorov-Smirnov normality test. The analysis, performed using SPSS 25.0, demonstrated that the viscosity data for formulations F1-F7 followed a normal distribution with a P-value of ≥0.05, indicating significance. Furthermore, the homogeneity test, conducted using the *Levene* Test, yielded a non-significant P-value ≥0.05, indicating that the data were homogeneous. Meeting the requirements of normality and homogeneity allowed for the continuation of the two-way comparison test, specifically, the two-way ANOVA test. Further analysis was carried out using the Between-Subjects Effects test to examine the effects of the formula and day. The obtained significance value of ≤0.05 indicated that both formula and day had a significant

impact on the viscosity data. Consequently, it was necessary to proceed with the ANOVA test to the *Post Hoc* test stage, which serves as a post-hoc test to assess the presence of significant differences between groups. can be found in **Table V**.

Table V. Hair Tonic Viscosity Test Combination of *Moringa* Leaf Extract and Fragrant Pandan Leaf Extract

Formula	Viscosity				
	Day- 0	Day- 7	Day-14	Day-21	Day-28 [bcde]
F1	1.66±0.01	1.66±0.01	1.69±0.01	1.66±0.00	1.70±0.00
F2	1.76±0.00	1.78±0.02	1.78±0.01	1.67±0.01	1.70±0.00
F3	1.70±0.00	1.71±0.00	1.72±0.00	1.69±0.00	1.69±0.00
F4	1.69±0.01	1.68±0.00	1.7±0.00	1.70±0.01	1.69±0.00
F5	1.79±0.01[a]	1.80±0.00	1.7±0.01	1.72±0.00	1.76±0.00
F6	1.81±0.01	1.82±0.01	1.81±0.01	1.70±0.00	1.70±0.00
F7	1.64±0.00	1.64±0.00	1.64±0.00	1.64±0.00	1.64±0.00

Notes:*a* = significantly different from F1, *b* = there is a significant difference in viscosity compared to day 0, *c* = there is a significant difference in viscosity compared to day 7, *d* = there is a significant difference in viscosity compared to day 14, *and e* = there is a significant difference in viscosity compared to day 21.

Specific gravity measurements were conducted from weeks 0 to 4 using a pycnometer at room temperature to assess the purity of the preparation, particularly in its liquid form. The specific gravity values of the preparation range from 0.97 ± 0.00 to 0.99 ± 0.00 g/mL, consistent with the principle that the specific gravity of Hair Tonic is lower than 1, which is the specific gravity of water (Darajati & Ambari, 2021). Based on the evaluation of the specific gravity results, it can be concluded that all formulated Hair Tonic preparations exhibited acceptable specific gravity. These findings demonstrate that the preparations align with the desired purity criteria, as indicated by the appropriate specific gravity values (Budastra *et al.*, 2023). Subsequently, statistical analysis was performed on the specific gravity data using the one-sample Kolmogorov-Smirnov normality test. The analysis, conducted using SPSS 25.0, revealed that the specific gravity data for formulations F1-F7 exhibited a significant normal distribution with a P-value of ≥0.05. Furthermore, the homogeneity test conducted using Levene's test yielded a significant P-value ≥0.05, indicating that the data were homogeneous. Meeting the requirements of normality and homogeneity allowed for the continuation of the two-way comparison test, specifically the two-way ANOVA test. are listed in **Table VI**.

Table VI. Hair Tonic Specific Gravity Test Combination of *Moringa* Leaf Extract and Fragrant Pandan Leaf Extract.

Formula	Specific Gravity Test (g/mL)				
	Day-0	Day- 7	Day-14	Day-21	Day-28
F1	0.98± 0.00	0.98± 0.00	0.98± 0.00	0.97 ± 0.00	0.98± 0.00
F2	0.97 ± 0.00[a]	0.98± 0.00	0.98± 0.00	0.98± 0.00	0.97 ± 0.00
F3	0.98± 0.00	0.97± 0.00	0.98± 0.00	0.99± 0.00	0.98± 0.00
F4	0.98± 0.00	0.98± 0.00	0.98± 0.00	0.98± 0.00	0.98± 0.00
F5	0.98± 0.00	0.98± 0.00	0.98± 0.00	0.99± 0.00	0.98± 0.00
F6	0.98± 0.00	0.99± 0.00	0.98± 0.00	0.98± 0.00	0.98± 0.00
F7	0.99± 0.00	0.99± 0.00	0.99± 0.00	0.99± 0.00	0.99± 0.00

Notes: [a] = significantly different from F7

The Rat Hair Growth Test was conducted to evaluate the efficacy of the Hair Tonic preparation formulation, which consisted of a combination of *moringa* leaf extract and fragrant pandan leaf extract.

Next, a statistical analysis was conducted to evaluate hair growth using the one-sample Kolmogorov-Smirnov test for normality in SPSS 25.0. The results of the normality test for mouse hair length indicated a significantly normal distribution, with a P-value ≥0.05. Additionally, the homogeneity test using Levene's test yielded a non-significant P-value ≥0.05, indicating that the data were homogeneous. Meeting the requirements of normality and homogeneity allowed for the continuation of the two-way comparison test, specifically, the two-way ANOVA test. In the Tests of Between-Subjects Effects for variations in formula and day of observation, a significance value of ≤0.05 was obtained, indicating a significant effect of both the formula and the observation day. Consequently, it was necessary to proceed with the ANOVA test to the *post-hoc* test stage, which serves as a further test to assess the presence of significant differences between groups. The analysis of mouse hair length is presented in **Table VII**. The results showed that hair length differed significantly from day 0 to day 28. These findings are consistent with the study conducted by (Maharini *et al*., 2023), where the average hair length did not exhibit significant differences. This lack of significance may be attributed to the behavior of the test animals, who frequently licked their backs after spray treatment, resulting in insignificant changes in the average hair growth observed on a weekly basis over the 28-day period (Maharini *et al*., 2023). This is supported by a study conducted by (Hindun *et al*., 2023), which emphasized that higher concentrations of treatment result in faster hair weight growth. This relationship can be attributed to the direct proportionality between the concentration of kelor leaf extract and the content of the active compounds. Kelor leaf extract (*Moringa oleifera*) is known for its significant benefits in scalp and hair care owing to its rich nutrient composition. It contains minerals, essential amino acids, and antioxidants, such as vitamin C, vitamin E, flavonoids, and tannins. Additionally, pandan leaves contain tannins, quinones, saponins, and flavonoids, which play a role in stimulating hair growth (Hindun *et al*., 2023). can be seen in **Table VII**.

Table VII. Results of Rat Hair Length Hair Tonic Preparation Formulation Combined With Ethanol Extract of *Moringa* Leaves and Ethanol Extract of Fragrant Pandan

Formula	Average Hair Length (mm) ± SD			
	Day-7 ^{jkl}	Day-14 ^{ikl}	Day-21 ^{ijl}	Day-28 ^{ijk}
F1	2.29± 0.15 ^{defgh}	4.51± 0.04	5.24± 0.10	6.43± 0.04
F2	3.22± 0.09 ^{efgh}	4.40 ± 0.04	5.50 ± 0.12	6.88 ± 0.04
F3	3.22± 0.05 ^{fgh}	4.45 ± 0.13	5.77 ± 0.24	6.92 ± 0.03
F4	4.43± 0.04^{abc}	4.93 ± 0.03	5.98 ± 0.00	6.96 ± 0.00
F5	4.38±0.00^{abc}	5.36 ± 0.04	6.36 ± 0.03	7.49± 0.06
F6	4.45±0.07^{abc}	5.44 ± 0.06	6.64 ± 0.02	7.49 ± 0.06
F7	4.15±0.06^{ab}	5.51±0.10	6.85±0.01	7.52±0.13
F8	3.89±0.01^{a}	5.81±0.03	6.87±0.04	7.53±0.08

Notes:

^{a}= there is a significant difference with F1, ^{b}= there is a significant difference with F2, ^{c}= there is a significant difference with F3, ^{d}= there is a significant difference with F4, ^{e}= there is a significant difference with F5, ^{f}= there is a significant difference with F5 ^{g}= there is a difference significant with F7 ^{h} = there is a significant difference with F8

[i]= there is a significant difference with day 7, [j]= there is a significant difference with day 14, [k]= there is a significant difference with day 21 [l]= there is a significant difference with day 28

Weight of rat Hair Tonic preparation formulation combined with ethanol extract of *Moringa* leaves and ethanol extract of fragrant pandan, and statistical data testing were conducted on rat hair weight using the one-sample Kolmogorov-Smirnov normality test in the SPSS 25.0 program. The results of the normality test for rat hair weight indicated a significant normally distributed distribution, with a P-value ≥0.05. Additionally, the homogeneity test was performed using Levene's test, which yielded a non-significant P-value ≥0.05, indicating that the data were homogeneous. Meeting the requirements of normality and homogeneity allowed for continuation of the one-way comparison test, specifically the one-way ANOVA test. In the Tests of Between-Subjects Effects for formula and day, a significance value of ≤0.05 was obtained, signifying that both the formula and the day had a significant effect. Therefore, it was necessary to proceed with the ANOVA test to the *post-hoc* test stage. The Post Hoc Test served as a follow-up test to assess significant differences between the groups. By conducting these statistical tests, we ensured that the rat hair weight data met the assumptions of normality and homogeneity, allowing for the effective analysis of the effects of the formula and observation day (Hindun *et al*., 2023).

The results of the rat hair weight test revealed that formulation 7 exhibited the highest hair weight among the groups, and a significant difference was observed compared to the positive control group treated with aloe vera, which demonstrated the best hair growth rate and hair weight. These findings suggest that treatment with a combination of 4% kelor leaf extract and 2% pandan leaf extract resulted in the most favorable hair weight growth rate. These results align with a study conducted by (Pratama *et al*., 2023), which reported that a combination of 1% kelor leaf extract and 1% rambutan leaf extract in a Hair Tonic formulation yielded an average hair length of 3 cm and hair weight of 0.236 g, representing the optimal concentration for stimulating hair growth activity. The positive control group treated with minoxidil 2.5% exhibited an average hair length of 2.025 cm and a hair weight of 0.207 g, with no statistically significant difference observed between the extract and the positive control minoxidil 2.5% (P <0.05). The detailed results are presented in **Table VIII**.

Table VIII. Hair Weight Results From A Hairtonic Formulation Using A Combination of *Moringa* Leaf Ethanol Extract and Fragrant Pandan Ethanol Extract

Formula	Mouse Hair Weight (g)
	H-30
F1	0.56 ± 0.05 [cdefgh]
F2	0.64 ± 0.03 [fgh]
F3	0.65 ± 0.03 [afgh]
F4	0.72 ± 0.08[abcde]
F5	0.75± 0.03[abcde]
F6	0.79 ± 0.03[abcd]
F7	0.67± 0.05[afgh]
F8	0.66± 0.01[afgh]

Notes: [a]= there is a significant difference with F1, [b]= there is a significant difference with F2, [c]= there is a significant difference with F3, [d]= there is a significant difference with F4, [e]= there is a significant difference with F5, [f]= there is a significant difference with F6 [g]= there is a difference significant with F7 [h] = there is a significant difference with F8

Flavonoids and saponins are believed to stimulate rat hair growth. Flavonoids are polar compounds that strengthen capillary walls, enhance blood flow to hair follicles, and promote the transition from the telogen to the anagen phase, thereby triggering hair growth.

In contrast, saponins function by creating foam that effectively cleanses the skin from dirt. Additionally, saponins can increase blood flow to the hair follicles. Insufficient blood flow to hair follicles can lead to hair loss (Maharini *et al*., 2023). Previous studies have detected flavonoid and saponin compounds in kelor and pandan wangi leaves. Kelor leaves also contain phytosterol compounds including brassicasterol, ergostadienol, methylene cholesterol, campasterol, campestanol, stigmasterol, ergostadienol, clerosterol, stigmastanol, avenasterol, stigmastadienol, isoavenasterol, and stigmastenol. These compounds, along with *β*-sitosterol, stigmasterol, and compasterol, are believed to possess properties that can prevent baldness and alopecia. Studies have shown that phytosterol content in plants, ranging from concentrations of 0.01% to 0.5%, has demonstrated significant anti-alopecia effects (Korassa *et al*., 2022).

CONCLUSION

Moringa leaf ethanol extract and fragrant pandanus leaf ethanol extract can be formulated in the form of *Hair Tonic* preparations that meet organoleptic, pH, viscosity, and specific gravity requirements. Differences in variations in the combination of *Moringa* and fragrant pandanus leaf extracts did not cause significant differences in the activity of rat hair length growth. The combination variation was not significantly different from that of the single extract of *moringa* and *fragrant pandanus* leaves. However, the single extract was significantly different from that of the normal control and negative control groups. The *Hair Tonic* activity of the combination variation was significantly different in terms of rat hair weight. The combination variation was significantly different from the single extract, and the single extract was significantly different from the normal and negative groups.

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