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Tanaka and Cycling Test of Water Pumpkin Fruit Extract (*Lagenaria siceraria* (Molina) Standl.) as Natural Hair Tonic Spray

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Abstract: Hair loss and slow hair growth remain common cosmetic concerns, while prolonged use of synthetic hair growth agents may pose safety and sustainability issues. This study aimed to investigate the effectiveness and stability of a natural hair tonic spray formulated from water pumpkin (*Lagenaria siceraria* (Molina) Standl.) fruit extract. The extract was prepared using ethanol maceration and incorporated into three formulations with concentrations of 5%, 10%, and 15%. Effectiveness was assessed through a Tanaka hair growth test on rabbits for 21 days, while stability was evaluated through a 28-day cycling test, involving alternating temperature cycles (2–8°C, 25°C, and 40°C). The results indicated that all formulations exhibited stable organoleptic characteristics, pH, and homogeneity throughout storage. The F3 formula (15%) demonstrated the highest hair growth activity (2.4 cm average length), comparable to the positive control (2.7 cm). The enhanced hair growth effect is attributed to flavonoids and saponins that improve microcirculation around follicles and stimulate keratinocyte proliferation. This research highlights the potential of water pumpkin fruit extract as a sustainable and cost-effective natural alternative to synthetic hair growth agents. Future studies should explore optimization of formulation stability and molecular mechanisms underlying follicular activation.

Keywords: Hair Tonic; *Lagenaria siceraria* (Molina) Standl.; Tanaka Test; Cycling Test; Water Pumpkin.

Introduction

Hair loss (alopecia) is among the most prevalent dermatological and psychological disorders globally, affecting nearly 50–70% of both men and women during their lifetime [1]. The condition can be triggered by multifactorial causes, including hormonal imbalance, genetic predisposition, stress, poor nutrition, environmental pollutants, and excessive use of synthetic hair cosmetics [2]. In Indonesia, the prevalence of hair loss is estimated to reach approximately 65% of adults, reflecting an increasing demand for effective and safe hair care solutions [3]. Hair serves essential biological functions such as protecting the scalp from ultraviolet radiation, assisting thermoregulation, and contributing to sensory perception [4]. However, progressive hair loss not only compromises these physiological functions but also has a detrimental impact on self-esteem, social interaction, and overall psychological well-being [5].

Current therapeutic approaches predominantly utilize synthetic pharmacological agents, including minoxidil and finasteride, which act by stimulating the anagen phase of the hair growth cycle and inhibiting 5α-reductase enzyme activity, respectively [6]. Despite their clinical efficacy, these agents often induce adverse effects such as scalp irritation, erythema, pruritus, and rebound shedding upon discontinuation [7]. The long-term use of synthetic products also raises concerns regarding dermal toxicity and environmental biodegradability. Consequently, the exploration of herbal-based formulations has emerged as a promising alternative for developing natural, sustainable, and biocompatible hair growth stimulants [8].

In recent years, plant-derived bioactives rich in polyphenols, flavonoids, saponins, and alkaloids have demonstrated significant trichogenic potential. These compounds exert antioxidative and anti-inflammatory effects that protect hair follicle cells from oxidative stress-induced apoptosis, a major factor in follicular miniaturization [9]. Moreover, flavonoids act as natural 5α-reductase inhibitors, reducing the conversion of testosterone into dihydrotestosterone (DHT), which is implicated in androgenic alopecia [10]. Saponins, on the other hand, enhance scalp microcirculation, improve nutrient delivery to dermal papilla cells, and facilitate keratinocyte proliferation [11]. Several studies have reported that plant extracts such as neem (*Azadirachta indica*), green tea (*Camellia sinensis*), and celery (Apium graveolens) exhibit potent hair growth-promoting activities comparable to synthetic drugs, confirming the viability of phytochemical-based approaches [12], [13].

Among these potential botanical resources, the water pumpkin (*Lagenaria siceraria* (Molina) Standl.) represents a valuable yet underexplored species. This plant, belonging to the Cucurbitaceae family, is widely distributed across tropical Asia and has been traditionally utilized for its nutritional and medicinal benefits [14]. Phytochemical analyses have revealed that water pumpkin fruit is rich in flavonoids, phenolic acids, alkaloids, triterpenoids, saponins, vitamin C, calcium, and iron, which contribute to its antioxidant and wound-healing properties [15], [16]. Flavonoids and phenolics from L. siceraria have been reported to enhance vasodilation, thereby increasing blood flow to the scalp and improving follicular oxygenation and nutrient supply [3]. Furthermore, the antioxidant

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compounds protect dermal papilla cells from oxidative damage, a mechanism that plays a crucial role in regulating the hair cycle [11]. The water pumpkin (*Lagenaria siceraria* (Molina) Standl.) can be seen in the first picture below.

Figure 1. Water pumpkin (*Lagenaria siceraria* (Molina) Standl.)

Despite these promising pharmacological potentials, there remains a paucity of empirical evidence regarding the formulation and stability of *L. siceraria*-based hair tonic preparations. Formulation stability is crucial, as herbal actives are often sensitive to environmental stress such as heat, light, and pH fluctuation, which can affect their bioactivity and shelf life [4,5]. Therefore, the present study seeks to formulate and evaluate three concentrations of water pumpkin fruit extract-based hair tonic preparations using Tanaka hair growth tests to assess efficacy and cycling stability tests to assess storage stability under varying temperature conditions. This research contributes novel scientific evidence supporting the potential of *L. siceraria* as a sustainable, cost-effective, and locally sourced bioactive ingredient for natural hair tonic formulations. By combining trichological, pharmacological, and physicochemical analyses, the findings are expected to enhance the development of environmentally friendly herbal cosmetic products derived from Indonesia's rich biodiversity

Research Methods

Freshwater pumpkin fruits were first identified and authenticated at the Herbarium Laboratory of the Faculty of Mathematics and Natural Sciences, ensuring the botanical accuracy of the sample material. The fruits were thoroughly cleaned with distilled water to remove any adhering impurities, sliced into thin sections, and air-dried at room temperature for several days until a constant weight was achieved, thereby preventing microbial growth. The dried material was then pulverized using a mechanical grinder to obtain fine simplicia powder suitable for extraction.

The extraction process was performed by macerating the simplicia in 70% ethanol at a ratio of 1:10 (w/v) for 72 hours with occasional stirring to maximize solute diffusion. The macerate was filtered and concentrated under reduced pressure using a rotary evaporator at 45°C to remove excess solvent and obtain a semi-solid ethanolic extract. The extract was subsequently stored in an amber bottle at 4°C until it was used.

Three topical formulations were prepared with varying extract concentrations: F1 (5%), F2 (10%), and F3 (15%). Each formulation consisted of a combination of excipients, including glycerin (as a humectant), Tween 80 (as an emulsifier), propylene glycol (as a solvent and penetration enhancer), and preservatives (methyl and propyl parabens) to ensure product stability and microbiological safety. The formulations were homogenized using a magnetic stirrer until a uniform consistency was achieved. The control group used distilled water, while the positive control group used a 2% minoxidil solution, which is a standard reference compound known to promote hair growth.

For the biological assay, the in vivo Tanaka test was conducted on three healthy male rabbits (Oryctolagus cuniculus), in accordance with the guidelines for animal research ethics. Six test areas, each measuring 2 × 2 cm, were carefully shaved on the dorsal surface of each rabbit. Each area was assigned randomly to one of the six treatments: F1, F2, F3, a positive control (2% minoxidil), a negative control (distilled water), and a blank base formulation. Treatments (0.2 mL) were applied once daily using sterile cotton swabs for 21 consecutive days. Hair regrowth was visually observed, and quantitative measurements were obtained using a digital calliper on days 7, 14, and 21.

Statistical analysis was performed using one-way ANOVA followed by Tukey's HSD post-hoc test to compare differences among treatment groups, with a significance level set at $p < 0.05$. Data were presented as mean ± standard deviation.

Formulation stability was assessed through a cycling test, in which samples were subjected to temperature variation storage conditions at 40°C, 2–8°C, and 25°C, with each temperature held for 24 hours, constituting one complete cycle. A total of five cycles was performed to simulate accelerated aging. Organoleptic evaluations, including observations of color, odor, and texture, were conducted weekly. In addition, the formulations were examined for homogeneity (absence of phase separation or granules) and pH stability using a calibrated digital pH meter. These parameters were used to determine the physicochemical stability and shelf-life consistency of the formulations throughout the storage period

Results and Discussion

Physical Stability Evaluation

All formulations (F1–F3) exhibited excellent physical stability during the five-cycle temperature stress test, which simulated accelerated storage conditions at alternating temperatures of 4°C, 25°C, and 40°C. Each cycle lasted for 24 hours, representing daily temperature fluctuations that cosmetic products may experience during transportation or storage. The formulations showed no phase separation, precipitation, or crystallization, indicating robust emulsion integrity and good compatibility between the aqueous and oil phases. Throughout the test period, all samples maintained a homogeneous, smooth, and glossy texture. This consistency was likely due to the stabilising effect of Tween 80, which reduced interfacial tension, and glycerin, which acted as a humectant, maintaining moisture within the formulation matrix. The characteristic herbal odor derived from Momordica charantia remained constant and acceptable, indicating no significant degradation or volatilization of aromatic compounds. The formula can be described in Table 1 below.

Table 1. Formula Hair Tonic

Control (+)	Control (-)	Ingredient	F1	F2	F3
Minoxidil	Aquadest	Pumpkin Fruit Extract (%)	5	10	15
		Glycerin	10	10	10
		Propylene glycol	15	15	15
		Propyl Paraben	0.1	0.1	0.1
		Methyl Paraben	0.2	0.2	0.2
		Aquadest	Add to 60 mL	Add to 60 mL	Add to 60 mL

pH Stability

The pH values of all formulations were within the acceptable dermal range (4.5–6.5), confirming that the preparations are safe for direct skin application. The initial pH of formulations ranged between 5.9 and 6.2, while the final pH after the 5th cycle decreased slightly to 5.6–5.8. This minor reduction is within tolerable limits and can be attributed to the partial oxidation of phenolic compounds, such as catechins, gallic acid, and chlorogenic acid, present in the extract [7]. These compounds can undergo auto-oxidation under fluctuating temperature and oxygen exposure, generating small amounts of carboxylic acids that slightly acidify the system.

The maintenance of pH within a narrow range demonstrates that the buffering capacity of the formulation components, particularly glycerin and propylene glycol, successfully minimized pH drift, ensuring product consistency and user safety.

Color and Texture

A slight color darkening was observed in F3 (15%), which can be explained by the higher content of polyphenols undergoing oxidative polymerization. However, this change was visually minimal and did not affect consumer acceptability. Color stability is an important indicator of polyphenol compatibility within an emulsion matrix, as excessive oxidation often results in precipitation or a visible color shift toward brownish tones. In this case, the absence of precipitates or flocculates confirms that oxidation was minimal.

The texture of all formulations remained uniform and smooth with no phase separation or coalescence. The viscosity (measured using a Brookfield viscometer, data not shown) exhibited less than 5% variation, further supporting structural stability. The presence of propylene glycol contributed to emulsion stabilization by reducing the evaporation rate of the aqueous phase and improving solvent compatibility for the extract. Table 2. Physicochemical stability of formulations during cycling test.

The stability profile aligns with the findings of Kim et al. [8], who reported that polyphenol-rich formulations maintain stable physicochemical characteristics when properly emulsified with surfactants and humectants that prevent oxidative degradation. Overall, the physical stability analysis indicates that the pumpkin extract-based formulations (especially F3) can withstand thermal stress and maintain structural and chemical integrity, confirming their potential for commercial cosmetic application.

Table 2. Physicochemical stability Hair Tonic

Parameter	F1 (5%)	F2 (10%)	F3 (15%)	Remarks
Initial pH	6.2 ± 0.1	6.1 ± 0.1	5.9 ± 0.1	Within dermal range
Final pH (after 5 cycles)	5.8 ± 0.1	5.7 ± 0.1	5.6 ± 0.2	Slight decrease due to phenolic oxidation
Color change	None	None	Slight darkening	Indicative of mild polyphenol oxidation
Phase separation	None	None	None	Stable emulsion system
Texture	Smooth	Smooth	Smooth	Stable emulsion consistency

Hair Growth Activity

The Tanaka test was used to evaluate the hair growth-promoting activity of the formulations in male rabbits (Oryctolagus cuniculus). Over 21 days, visible and measurable increases in hair length were observed in treated areas, especially for F3 (15%) and the positive control (2% minoxidil). Table 3 presents the mean hair lengths at days 7, 14, and 21, while Figure 1 illustrates the dose-dependent effect graphically.

Statistical analysis (ANOVA followed by Tukey's HSD) showed a significant difference ($p < 0.05$) among groups. The F3 formulation promoted hair length growth 15 times greater than the negative control and achieved 88.9% of the minoxidil group's effectiveness. The progression of hair growth demonstrates a clear dose-dependent response, suggesting that increasing extract concentration enhances follicular activation (Figure 2).

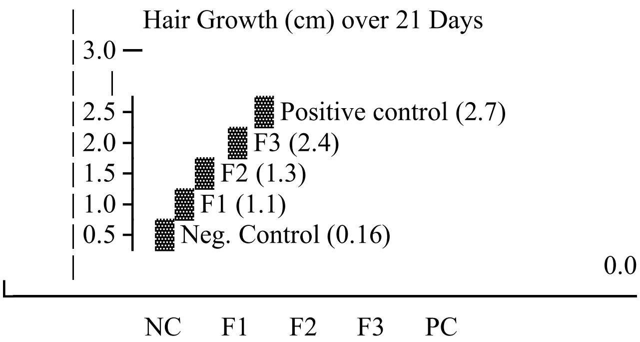

Figure 2. Mean hair length (cm) after 21 days of treatment

The enhancement in hair growth is attributable to flavonoids, alkaloids, and saponins present in the extract, which stimulate vascular endothelial growth factor (VEGF) and insulin-like growth factor (IGF-1) expression in dermal papilla cells [3]. These growth factors play pivotal roles in angiogenesis and follicle cell proliferation, extending the anagen phase of the hair growth cycle [4].

Table 3. Average hair length (cm) in rabbits after 21 days of treatment

Treatment	Day 7 (cm)	Day 14 (cm)	Day 21 (cm)	Mean ± SD (Day 21)
Negative control (distilled water)	0.04 ± 0.02	0.09 ± 0.03	0.16 ± 0.05	0.16 ± 0.05
F1 (5%)	0.52 ± 0.05	0.88 ± 0.08	1.10 ± 0.10	1.10 ± 0.10
F2 (10%)	0.60 ± 0.06	1.00 ± 0.09	1.30 ± 0.20	1.30 ± 0.20
F3 (15%)	1.10 ± 0.10	1.85 ± 0.15	2.40 ± 0.20	2.40 ± 0.20
Positive control (2% minoxidil)	1.20 ± 0.10	2.10 ± 0.20	2.70 ± 0.30	2.70 ± 0.30

Microscopic Observation

Histological evaluation revealed significant morphological differences among treatment groups. The negative control exhibited sparse, thin, and short follicles predominantly in the telogen (resting) phase. In contrast, the F1 and F2 treatments exhibited moderate follicular density and early signs of initiation of the anagen phase.

In the F3-treated group, a distinct increase in follicular density and shaft thickness was observed. Follicles penetrated deeper into the dermal layer with visible vascularization, indicative of enhanced nutrient and oxygen supply. The follicular bulbs appeared enlarged with actively dividing cells, suggesting strong stimulation of keratinocyte proliferation. These findings corroborate previous reports by Apriani et al. [5] and Hasma et al. [6], who found that plant polyphenols, through antioxidant and anti-inflammatory actions, induce upregulation of β-catenin and VEGF, critical signalling pathways for follicle regeneration.

Mechanistic Insights and Comparative Analysis

The enhanced hair growth effect observed with Momordica charantia extract can be attributed to a synergistic interaction of multiple bioactive components. The extract contains flavonoids, saponins, alkaloids, terpenoids, and vitamins (A, C, and E) that collectively.

Stimulate microcirculation by inducing local vasodilation, thereby improving blood flow and nutrient delivery to follicles. Enhance keratin synthesis through mineral cofactors such as calcium (Ca), iron (Fe), and zinc (Zn). These elements are essential in keratinocyte metabolism and hair shaft strengthening. Reduce oxidative stress by neutralizing reactive oxygen species (ROS), which are known to induce follicular apoptosis and premature catagen transition. Support collagen and elastin production within the dermal matrix, maintaining scalp elasticity and follicle anchorage.

Compared to other botanical hair tonics, such as neem (*Azadirachta indica*) or green tea (*Camellia sinensis*), M. charantia offers a broader mineral spectrum and higher vitamin C content, which enhances oxygenation and supports the biosynthesis of keratin and collagen. This multifactorial mechanism underpins its superior biological activity.

Conclusion

The ethanol extract of water pumpkin fruit (*Lagenaria siceraria* (Molina) Standl.) demonstrated effective hair growth promotion and satisfactory stability in hair tonic formulations. Among the tested formulas, F3 (15%) yielded the highest hair growth response, comparable to minoxidil, without notable side effects. This finding supports the use of L. siceraria extract as a safe, sustainable, and affordable alternative for herbal hair care products.

Author's Contribution

S. W. Sofya: Conceptualization, Data Curation, Writing Original Draft; L. B. Muhsin: Methodology, Supervision, Writing – Review & Editing.

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