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Formulation and in Vitro Efficacy Evaluation of Polyherbal Hair Tonics for Enhancing Hair Health on Various Hair Types

Nur Amalina Aliudin[1], Sheba Rani David[1,2] and Rajan Rajabalaya[1]*

[1]PAPRSB Institute of Health Sciences, Universiti Brunei Darussalam, Jalan Tungku, Bandar Seri Begawan, Brunei-Muara, Brunei Darussalam.
[2]School of Pharmacy, University of Wyoming, Laramie, Wyoming, USA.
*Corresponding author E- mail: rajan.rajabalaya@ubd.edu.bn

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Hair tonics are commonly used in hair care to improve hair health and manageability through natural ingredients. This study aimed to develop and evaluate polyherbal hair tonic formulations containing white tea, green tea, and cherry blossom extracts, assessing their physicochemical properties, stability, and efficacy in enhancing hair texture, thickness, and manageability. Two formulations were prepared: Hair Tonic A (10% white tea extract) and Hair Tonic B (10% green tea extract). The formulations were analyzed for pH, viscosity, density, and UV-Vis transmittance. Stability testing was conducted at room temperature (25°C), elevated temperature (50°C), and high humidity (75% RH, 40°C). In vitro efficacy tests on human hair tresses (black, blonde, and mixed) in both non-bleached and bleached states evaluated changes in hair thickness, weight, anti-frizz properties, and curl retention. Hair Tonic A demonstrated superior stability, maintaining a pH of 4.78, viscosity of 5.03 mPa/s, and density of 1.014 g/ml, with a UV-Vis transmittance of 88.66%. In contrast, Hair Tonic B showed instability under high temperatures and light exposure, with color changes and odor development by Day 28. Hair Tonic A increased hair thickness and improved manageability, particularly in blonde and black hair, while also showing better anti-frizz and curl retention properties. These findings suggest that the polyherbal formulations, especially Hair Tonic A, offer a promising daily-use hair care solution, with significant benefits in enhancing hair health and stability.

Keywords: Hair care formulations; Hair tress evaluation; Polyherbal hair tonic; Stability testing; White tea extract.

Hair is a prominent physical feature found in all mammals, including humans, where it serves several important biological and cosmetic functions. In humans, hair primarily acts as a protective layer, safeguarding the skin from external insults such as ultraviolet radiation, chemicals, and physical injuries. Additionally, it enhances the skin's perception of physical stimuli and facilitates thermoregulation, helping to maintain body temperature by controlling heat loss through the scalp [1]. Beyond these biological roles, hair is considered a significant cosmetic asset. Its appearance is associated with traits such as boldness, youth, and social status, which makes it an integral part of an individual's identity and self-expression [2,3].

The structure and properties of hair differ significantly across ethnic groups, influenced by variations in follicle shape and hair fiber composition. These variations are largely

due to differences in hair follicle shape and hair fiber ellipticity, which lead to differences in physicochemical properties such as color, appearance, mechanical resistance, and surface reactivity to chemical agents [2]. European hair is typically oval in cross-section, leading to straight or wavy hair, while Asian hair tends to be rounder, resulting in straighter hair. African American hair is generally more elliptical, producing tightly curled or coiled hair, which has unique mechanical properties and moisture retention characteristics [4].

The cosmetic industry has responded to these varying hair needs by developing a wide range of hair care formulations, including creams, lotions, powders, oils, shampoos, and conditioners [5]. Among these, hair tonics have gained popularity as a non-greasy liquid formulation designed to style and nourish hair. Hair tonics present multiple benefits compared to other hair care formulations, such as being lightweight, non-greasy, and easily absorbed by the scalp. . These formulations allow for transappendageal absorption, where active ingredients penetrate through hair follicles and sebaceous glands, enhancing efficacy [6]. Additionally, hair tonics do not leave residue on the scalp, reducing the risk of irritation and providing a pleasant, lightweight feel after use [7].

A key differentiator between hair tonics and other styling products, such as pomades, mousse, creams, and gels, is their inclusion of ingredients like alcohol and astringents. These components make the formulation lightweight and quick-drying, allowing hair tonics to be particularly suitable for individuals seeking to achieve a polished, well-groomed appearance without the greasy finish commonly associated with oil-based products [8]. Hair tonics can help hold hairstyles in place, enhance shine, and provide additional benefits, such as promoting scalp health by moisturizing dry scalps, removing product buildup, and unclogging hair follicles [9]. Furthermore, hair tonics offer protection against frizz, balance scalp pH, and add gloss to the hair, making them multifunctional in their approach to hair care [10].

In recent years, there has been growing interest in polyherbal formulations for hair care, driven by the rising demand for natural and sustainable ingredients. Polyherbal formulations combine extracts from multiple plants, each offering unique therapeutic benefits. Herbal extracts are rich in bioactive compounds such as antioxidants, vitamins, and amino acids that promote hair health by nourishing the scalp, strengthening hair fibers, and protecting against environmental damage. For instance, green tea extract, a popular ingredient in hair tonics, is known for its high content of catechins, which exhibit antioxidant properties that protect hair from oxidative stress caused by free radicals [11]. Similarly, white tea extract contains flavonoids and polyphenols that offer both anti-inflammatory and antioxidant benefits, making it an excellent choice for scalp care and hair strengthening [12].

Hair tonics with natural oils, such as coconut and almond oil, are also gaining popularity due to their ability to nourish and hydrate the scalp. Coconut oil is known for its high lauric acid content, which enhances its penetration into hair fibers, providing deep conditioning and reducing protein loss in damaged hair [13]. Almond oil, rich in essential fatty acids and vitamins, helps to repair dry, brittle hair and improve overall hair strength [14]. The integration of these ingredients into polyherbal hair tonic formulations not only improves the hair's physical appearance but also targets specific hair concerns such as dryness, split ends, frizz, and scalp inflammation [15].

Despite the growing popularity of polyherbal hair care products, there is a need for scientific evaluation of their efficacy and stability. While many studies have focused on the individual benefits of specific herbal extracts, there is limited research on the optimization of polyherbal formulations and their effects on different hair types. Factors such as hair thickness, porosity, and chemical treatment history can significantly influence how a formulation performs on different individuals [16]. Additionally, the stability of herbal formulations is a critical consideration, as the natural ingredients may be prone to degradation when exposed to light, temperature fluctuations, or humidity, potentially reducing the product's shelf life and effectiveness [17].

The objectives of this study are to prepare and optimize the formulation of polyherbal hair tonics using extracts such as white tea, green tea, and cherry blossom. The formulated tonics will be evaluated for their physicochemical properties, including pH, viscosity, density, and stability, under

different environmental conditions. In addition, the efficacy of the hair tonics will be assessed through in vitro testing on human hair tresses of varying types (black, blonde, and mixed). Key performance indicators such as hair thickness, weight, anti-frizz properties, and curl retention will be measured to determine the effectiveness of the formulations in improving hair health and manageability.

The primary objective of this study was to formulate and evaluate polyherbal hair tonic preparations incorporating white tea, green tea, and cherry blossom extracts. The study aimed to assess the physicochemical properties, stability, and in vitro efficacy of the formulations on different hair types (black, blonde, and mixed hair). Specific evaluations included pH, viscosity, density, UV-Vis transmittance, and stability under varying environmental conditions. Additionally, the study sought to investigate the formulations' ability to improve hair thickness, texture, anti-frizz properties, and curl retention. The overarching goal was to develop multifunctional, natural hair care products that promote both hair and scalp health, while addressing common hair concerns such as frizz, dryness, and damage, with a focus on formulating stable and effective solutions suitable for commercial adaptation.

MATERIALS AND METHODS

Chemicals and Reagents

White Tea extract, Green Tea extract, Sakura Cherry Blossom Extract, ProCutiGen® Thermal Shield, FSS Keratin Hydrolysate 30PF, FSS Kera Straightening & Curl, FSS White Tea Extract PF, Active Lite® Relaxer, and Propane-1,3 diol were procured from the Formulator Sample Shop, located in Bareggio, Milan, Italy. Menthol Crystal and Ethyl Alcohol were sourced from Merck, Germany. All materials used in the preparation of the polyherbal hair tonic formulations were of analytical grade, ensuring the consistency and accuracy required for formulation development and subsequent evaluation.

Preparation of Polyherbal Hair Tonic

Two final polyherbal hair tonic formulations were developed: Hair Tonic 1, containing White Tea extract, and Hair Tonic 2, containing Green Tea extract (Table 1). Initially, three plant extracts, including White Tea, Green Tea, and Sakura Cherry Blossom, were trialed for the formulations. Each formulation incorporated two concentrations (5% and 10%) of the herbal extracts, which were dissolved with appropriate surfactants and preservatives. Ultra-pure distilled water was used as the base vehicle for both formulations. The varying concentrations of the polyherbal ingredients were tested to optimize the formulations while maintaining desirable organoleptic properties, such as physical appearance and odor.

ProCutiGen® Thermal Shield, FSS Keratin Hydrolysate, and other active components were incorporated step-by-step to ensure proper integration and formulation stability. Finally, ultra-pure water was added to reach the desired volume, and the solution was transferred into a clear spray bottle. Similarly, Hair Tonic 2 was prepared by the stepwise addition of ProCutiGen® Bond, FSS Keratin Hydrolysate 30PF, FSS Kera Straightening & Curl, FSS Green Tea Extract PF, and Active Lite® Relaxer to Propane-1,3 diol. The dissolved Menthol Crystal in Ethyl Alcohol was then combined with the polyherbal mixture, followed by the addition of water to complete the formulation. The trial formulation using Sakura Cherry Blossom Extract was prepared using the same method as Hair Tonic 2, with FSS Green Tea Extract PF replaced by Pure Natural Sakura Cherry Blossom Extract. The sequential addition of active ingredients and careful formulation optimization ensured stability and efficacy in the resulting hair tonic formulations [3]. (**Table 1**).

Characterization methods of polyherbal preparations

All hair tonic formulations were subjected to characterization studies, including pH, viscosity, density, stability testing, and transmittance measurements. During the stability test, the hair tonics were evaluated for their organoleptic properties under varying conditions. The organoleptic properties observed during the stability testing included color, surface appearance, clarity, texture, aroma, and intensity of odor. In this study, two hair tonic formulations containing 10% (w/w) White Tea and 10% (w/w) Green Tea were selected for comparison. The stability of these formulations was compared to assess

differences, as there has been increasing interest in the development of skincare products infused with tea extracts [11].

pH determination

The pH values of the hair tonics were measured at room temperature (25°C) using a pH meter (FiveEASY pH-meter, Mettler Toledo Instruments, Columbus, OH, Greifensee, Switzerland) [18]. The pH of each hair tonic was determined in triplicate, and the average values were recorded.

Density

The density was measured using a pycnometer or specific gravity bottle method at room temperature. The weight of the empty pycnometer or specific gravity bottle (W0) and the weight of the pycnometer or specific gravity bottle filled with the hair tonic sample (W1) were measured [18]. The volume of the hair tonic used were also recorded (V).

Density formula: (W1-W0) / V
Relative density: (W1-W0) / V × 0.997[19]

Viscosity

The viscosity values of distilled water and hair tonic were measured using Ostwald's viscometer (Calibrated Size A and Size D) [19]. The higher the viscosity of the liquid sample, the greater the flow resistance. The formula for relative viscosity (mPa) is as follows, where ž is the dynamic viscosity, K is the constant value of the instrument used, ñ is the density (g/cm³), and t is the time taken for the liquid to flow through the tube:

Ŋrel = (η) / (η (water) = Kρt / (Kρt (water)[19]

UV- Vis Transmittance measurement

The light transmittance of the polyherbal hair tonic was measured using a UV/Vis spectrophotometer (Shimadzu UV-1900i, Japan). This method allows for the evaluation of the clarity and optical properties of the formulation by measuring the amount of light passing through the sample [9].

Fourier-transform infrared spectroscopy (FTIR)

Fourier-transform infrared spectroscopy (FTIR) was conducted using a Shimadzu FTIR Plastic Analyzer (Japan) to analyze the functional groups in the liquid hair tonic formulations. A small volume of each sample was placed directly on the attenuated total reflectance (ATR) crystal, and the spectrum was recorded in the mid-infrared region (4000–400 cm{ [1]). Background scans were taken for accuracy, and spectra were compared to reference data to identify key functional groups present in the formulations. This method allows direct analysis of liquid samples without additional preparation [20].

In vitro efficacy tests of hair tonic

In vitro efficacy tests were conducted on human hair tresses (black, blonde, and mixed) to assess the impact of the polyherbal hair tonics on hair thickness, anti-frizz properties, curl retention, and manageability. Both non-bleached and bleached hair types were used to evaluate the formulations' performance on chemically treated and untreated hair. Each hair tress was treated with 0.74 g (5 pumps) of hair tonic spray. Hair Thickness Measurement: Hair thickness was measured before and after 24 hours of treatment using a digital vernier caliper. Measurements were recorded for all hair types to observe changes in thickness due to treatment. Anti-Frizz and Anti-Static Testing: Hair tresses were combed with a fine-tooth comb, and the length of the combed section was recorded. Anti-frizz performance was evaluated by comparing combed hair length before and after the application of the hair tonic. Curl Retention: Curls were created on the hair tresses using curling tools. After drying, the hair was placed in a humidity chamber at 75% RH and 40°C. Curl retention was measured at intervals (0, 90 minutes, 5 hours, and 24 hours) to assess the ability of the hair tonic to maintain curl under humid conditions.

Hair thickness properties

The diameter (mm) of the hair was measured before treatment (t = 0 hr) and after 24 hours (t = 24 hr) using a digital vernier caliper (Fisher Scientific Electronic Digital Caliper 0-150mm, Pittsburgh, USA) [21]. In the efficacy test, a standard dose of 5 pumps (0.74 g) of hair tonic spray was applied to each hair tress in both the negative control (non-bleached hair) and positive control (bleached hair) groups.

Anti-frizz and Anti-static analysis

A fine-tooth comb was used to brush the hair, and the length of the combed section was

measured before the hair became entangled. The procedure was performed by the same person to ensure consistent brush strokes, strength, and rhythm. Two pumps of the hair tonic were applied to the hair tresses in a downward motion. The hair was then combed ten times, and the results were photographed for documentation [22].

Curl retention analysis

Curls were created on the hair tresses by lightly wetting them with water, followed by the application of the hair tonic. The tresses were then rolled onto curling tools of the same size. Once the hair was dry, the curlers were removed, and the curled tresses were placed in a humidity chamber (75% RH, 40°C ± 2°C). The length of the curls (mm) was monitored at 0 hours, 90 minutes, 5 hours, and 24 hours [23].

Stability studies

The stability testing of the polyherbal hair tonics was conducted under various environmental conditions to evaluate their durability and efficacy over time. The formulations were stored at the following conditions: Room temperature (25°C ± 2°C); Elevated temperature (50°C ± 2°C in a drying chamber); High humidity (75% ± 5% RH at 40°C ± 2°C in a humidity chamber); With sunlight exposure (25°C ± 2°C in a light-exposed area); Without sunlight exposure (25°C ± 2°C in a dark area). Each hair tonic was observed over a 4-week period with assessments on Days 14 and 28. The formulations were evaluated based on their organoleptic properties, including color, odor, clarity, surface appearance, and texture. Additional assessments included measurements of pH, viscosity, and density under each condition to monitor for changes in physical properties that would indicate instability.
[24].

Statistical analysis

The data were statistically analyzed using GraphPad Prism software, Version 7. The numerical values for each group were expressed as mean ± standard deviation (SD) and subjected to an unpaired t-test. In all cases, a p-value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The percentage of components in the hair tonic formulations was determined using a formula optimization method. Three sets of trial formulations, each containing two different concentrations (5% and 10%) of herbal ingredients, were prepared. The lower concentration (5%) resulted in precipitation of the hair tonic at room temperature. The third formulation, which contained Sakura Cherry Blossom Extract, was not included in the final study. This study compares the differences between the hair tonic formulations containing tea extracts: White Tea (WT) extract and Green Tea (GT) extract (Table 1).

Physicochemical properties

The pH values of the two hair tonic formulations were 4.78 for HT A and 4.25 for HT B (Table 2). The viscosity of HT A was 5.03 mPa/s, while HT B measured 4.87 mPa/s, relative to water (ž = 1 mPa/s). The density of HT A was 1.014 g/ml, slightly higher than that of HT B (ñ = 1.007 g/ml). The UV/Vis transmittance percentage for HT A was also higher than HT B, at 88.66% and 75.38%, respectively.

Hair products must be formulated within an acceptable pH range to prevent irritation and itching of the scalp. Both Hair Tonic A and B formulations fall within the safe pH range for hair care products (pH 3.5–5.5) [25]. A hair tonic containing 2.5% Green Tea extract had a higher pH compared to HT B, which contains 10% Green Tea extract. The relationship between pH and Green Tea extract concentration is linked to the phenolic acid content present in the extract [8]. A higher concentration of Green Tea extract increases phenolic acid, resulting in a lower pH [26]. The viscosity of HT A (5.03 mPa/s) and HT B (4.87 mPa/s) are higher than that of water (ž = 1 mPa/s). Other studies have reported much higher viscosity values for their hair tonics (125 mPa/s and 140 mPa/s) due to the use of thickening agents like Glycerin and Carbopol® 940 [27]. In contrast, hair tonics containing 2.5%, 5.0%, and 7.5% Green Tea extract had viscosities of 0.228 mPa/s, 0.235 mPa/s, and 0.237 mPa/s, respectively [8]. This supports the higher viscosity of HT B (4.87 mPa/s) due to its 10% Green Tea extract content. The higher ethanol content (75%) in Amin's formulations contributed to their lower viscosities [8], while HT B only contains 10% Ethanol (70%), which explains its higher viscosity.

The density values of HT A (1.014 g/ml) and HT B (1.007 g/ml) are close to the density of

Table 1. Ingredients and percentage of ingredients in the hair tonic formulations

Name of product	Ingredient (INCI Name)	Formulation code	
		HT A (% w/w)	HT B (% w/w)
ProCutiGen® Thermal Shield	Hydrolyzed Keratin, Leuconostoc/Radish Root Ferment Filtrate	10.0	-
FSS Keratin Hydrolysate 30PF	Aqua, Hydrolyzed Keratin, Leuconostoc Filtrate	5.0	5.0
FSS Kera Straightening & Curl	Hydrolyzed Keratin & Trametes Versicolor Extract & euconostoc/Radish Root Ferment Filtrate	10.0	10.0
FSS White Tea Extract PF	Aqua & Camellia Sinensis Leaf Extract & Leuconostoc/Radish Root Ferment Filtrate	10.0	-
Active Lite® Relaxer	Polyquaternium-80 & Aqua & Pisum Sativum (Pea) Peptide & Selaginella Lepidophylla Extract & Sclerocarya Birrea Fruit Extract	10.0	10.0
FSS Green Tea Extract PF	Aqua & Camellia Sinensis Leaf Extract & Leuconostoc/Radish Root	-	10.0
ProCutiGen® Bond	Salvia Hispanica Seed Extract	-	10.0
ZEMEA	Propane-1,3-diol	5.0	5.0
L-Menthol	Menthol	0.2	0.2
Ethanol absolute	Alcohol	10.0	10.0
Ultra-pure water	Aqua (Water)	39.8	39.8

Abbreviation: W/W - weight by weight, INCI: International Nomenclature of Cosmetic Ingredients; Hair Tonic Formulation A (White Tea) & Hair Tonic Formulation B (Green Tea)

water. However, lower densities (0.884–0.896 g/ml) in their formulations, which can be attributed to their higher ethanol content (75% Ethanol 96%) compared to HT B, which contains a lower ethanol percentage. The percentage of UV light transmittance, as determined by the UV/Vis spectrophotometer for HT A and HT B formulations, is 88.66% and 75.38%, respectively. These results reflect the transparency of the hair tonic products. The HT A formulation is more transparent with minimal light scattering. In contrast, the lower percentage of light transmittance in the HT B formulation indicates that, while still transparent, it exhibits more light scattering [28].

Fourier-transform infrared spectroscopy (FTIR)

The FTIR spectra of the Hair Tonic A formulation and its main ingredients (ProCutiGen® Thermal Shield and FSS White Tea Extract) were analyzed across the range of 400 – 4000 cm{ ¹. The spectra for both ProCutiGen® Thermal Shield and White Tea Extract closely resemble that of Hair Tonic A, with the strongest similarity observed between White Tea Extract and Hair Tonic A. For example, peaks were identified between 1648 – 1638 cm{ ¹ and 3400 – 3300 cm{ ¹ in all three samples: Hair Tonic A, ProCutiGen® Thermal Shield, and White Tea Extract. Additionally,

Table 2. Evaluation of hair tonic formulations

Evaluation parameters	Hair Tonic Formulation	
	HT A	HT B
pH	4.78 ± 0.01	4.25 ± 0.02
Relative viscosity (mPa/s)	5.03	4.87
Dynamic viscosity (mPa/s)	3.70	3.59
Density (g/ml)	1.014	1.007
Relative density or Specific gravity (g/ml)	1.011	1.004
UV/Vis Transmittance Measurement (%)	88.66 ± 0.031	75.38 ± 0.093

Results show the pH, relative viscosity, dynamic viscosity, density and relative density values for two hair tonic formulations (HT A and HT B) *(n = 3).* Abbreviation: HTA – Hair Tonic A; HTB – Hair Tonic B

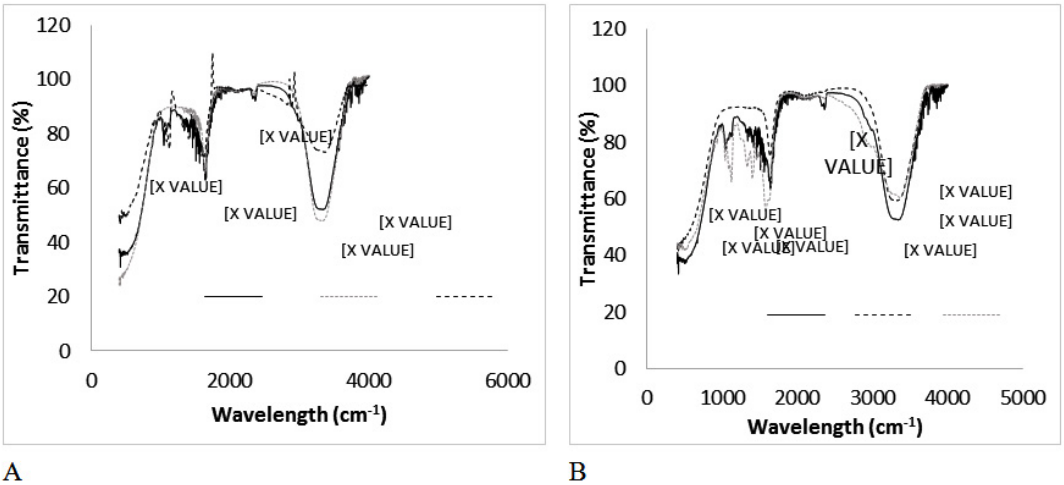

Fig. 1. FTIR spectra of Hair Tonic B formulation and its main ingredients at 400 – 4000 cm^{-1}; Hair Tonic B formulation with FSS Green Tea Extract PF and ProCutiGen® Bond.

ProCutiGen® Thermal Shield and Hair Tonic A produced similar transmittance measurements in the 1070 – 1030 cm{ ¹ range. Similarly, the FTIR spectra of Hair Tonic B, Green Tea Extract, and ProCutiGen® Bond were found to be closely aligned. Peaks in the 3400 – 3300 cm{ ¹ range were observed in all three samples, indicating the presence of N-H bonds due to aliphatic primary amines. Additionally, ProCutiGen® Bond and Hair Tonic B showed peaks between 1400 – 1000 cm{ ¹, corresponding to O-H bending and C=O stretching, likely due to secondary alcohols and phenols.

As shown in Figure 1A, the shared transmittance measurements between ProCutiGen® Thermal Shield and Hair Tonic A in the 1070 – 1030 cm{ ¹ region suggest the presence of S=O bonds, indicating a sulfoxide compound [29]. This finding aligns with a studies reporting that UV irradiation in FTIR analysis caused keratin breakdown, leading to an increase in oxidized sulfur [30,31]. Another significant peak was detected between 1648 – 1638 cm{ ¹ for Hair Tonic A, ProCutiGen® Thermal Shield, and White Tea Extract, indicating the presence of C=C bonds, attributed to polysaccharides in White Tea Extract In the 3400 – 3300 cm{ ¹ range, bands were observed, indicating N-H stretching, likely due to aliphatic primary amines found in White

Table 3. Hair diameter of different hair types before and after treatment with hair tonic (HT A formulation) at 0hr, 2hr, 4hr and 24hr

	Before treatment (t= 0hr)			After treatment (t= 24hr)		
	Diameter (mm)					
Type of hair	Non-bleached (with treatment)	Bleached (control)	Bleached (with treatment)	Non-bleached (with treatment)	Bleached (control)	Bleached (with treatment)
Black	0.04 ± 0.01	0.03 ±.0.01	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01
Blonde	0.04 ± 0.01	0.04 ± 0.03	0.04 ± 0.01	0.07 ± 0.01	0.04 ± 0.01	0.06 ± 0.02
Mixed (black and blonde)	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.00	0.03 ±.0.01	0.03 ± 0.02	0.04 ±.01

Table 3 illustrates the hair diameter for different hair types; black, blonde and mixed (black and blonde) as determined by Electronic Digital Caliper (n=3). Data shown as mean ± standard deviation of triplicate readings.

Table 4. Weight of different hair types treated with Hair Tonic (HT A formulation) at 0hr, 2hr, 4hr and 24hr

Time (hr)	Type of hair	Non-bleached (with treatment) (g)	Bleached (control) (g)	Bleached (with treatment)
(g)				
0	Black	18.27 ± 0.01	28.28 ± 0.02	28.28 ± 0.02
	Blonde	11.36 ± 0.01	11.59 ± 0.01	12.00 ± 0.02
	Mixed (black and blonde)	11.71 ± 0.00	11.56 ± 0.00	12.07 ± 0.00
2	Black	18.20 ± 0.01	28.41 ± 0.01	28.47 ± 0.01
	Blonde	11.45 ± 0.00	11.60 ± 0.01	12.15 ± 0.01
	Mixed (black and blonde)	11.73 ± 0.01	11.45 ± 0.00	11.92 ± 0.00
4	Black	18.14 ± 0.00	28.31 ± 0.01	28.37 ± 0.00
	Blonde	11.41 ± 0.00	11.56 ± 0.00	12.15 ± 0.01
	Mixed (black and blonde)	11.70 ± 0.01	11.42 ± 0.00	11.88 ± 0.00
24	Black	18.25 ± 0.00	28.64 ± 0.00	28.68 ± 0.00
	Blonde	11.50 ± 0.00	11.70 ± 0.00	12.15 ± 0.00
	Mixed (black and blonde)	11.80 ± 0.00	11.59 ± 0.00	12.05 ± 0.00

Tea Extract, which contains proteins and amino acids [32].

Figure 1A shows a strong similarity in the spectra between Hair Tonic A, ProCutiGen® Thermal Shield, and White Tea Extract, while Figure 1B highlights similarities between Hair Tonic B, Green Tea Extract, and ProCutiGen® Bond. In ProCutiGen® Bond, the peaks between 1400 – 1000 cm{ ¹ suggest the presence of O-H bending and C=O stretching, likely due to secondary alcohols and phenols. The peak observed between 3400 – 3300 cm{ ¹ corresponds to the presence of N-H bonds from aliphatic primary amines in ProCutiGen® Bond, Green Tea Extract, and Hair Tonic B. ProCutiGen® Bond contains Salvia Hispanica Seed Extract, which is rich in fatty acids (á-linolenic acid), monosaccharides (glucose), organic acids, and free amino acids [33]. The FTIR analysis provides strong evidence of the chemical similarities between the hair tonic formulations and their key ingredients, particularly in the regions associated with S=O, C=C, and N-H bonds, further confirming the composition and interactions of the active compounds. The presence of primary amines and polysaccharides is also well-supported by the results.

In-vitro evaluation test

Hair Thickness

Based on Table 3, there was an increase in thickness in all bleached hair types. Among non-bleached hair, only blonde hair showed a significant increase in thickness after treatment, from 0.04 mm to 0.07 mm. No observable difference in thickness was noted for non-bleached black or mixed (black and blonde) hair tresses before and after hair tonic treatment. According to the two-way ANOVA statistical analysis (Friedman and Dunn's multiple comparison tests), the difference in hair thickness between untreated and treated hair was statistically insignificant for all hair types: blonde ($p = 0.50$),

Table 5. Results of sliding comb in strands after 1 combing stroke in Anti-Frizz analysis

Type of hair	Non-bleached (With treatment) (cm)	Bleached (Control)(cm)	Bleached (With treatment) (cm)
Black	21.1	8.0	11.8
Blonde	48.5	14.7	29.2*
Mixed (black and blonde)	14.7	6.2	7.0*

Table 10. shows the length of combed hair for all air types; black, blonde and mixed (black and blonde). Asterisk (*) represents significant differences as compared to the negative control (* for p-value < 0.05, ** for p-value <0.01 and *** for p-value < 0.001), as determined by two-way ANOVA with Friedman's and Dunn's multiple comparison tests.

Fig. 2. Results of sliding comb in strands before and after treatment with Hair Tonic A, followed by 10 combing strokes.

mixed (p = 0.6667), and black hair tresses (p = 0.6667).

As indicated in Table 3, only non-bleached blonde hair exhibited a significant increase in thickness after treatment with HT A formulation. No noticeable change in thickness was observed in non-bleached black or mixed hair tresses. The difference in hair thickness after application is attributed to lower product retention on dark-coloured hair, which has low porosity. The increase in thickness for all bleached hair types after treatment is likely due to their higher porosity, as bleaching erodes layers of the hair cuticle [34]. The higher porosity allows the hair product to be absorbed more readily into the hair shaft, rather than remaining on the surface, making the product more effective on porous hair [35]. This suggests that hair tonics perform better on bleached or more porous hair, enhancing product absorption and effectiveness.

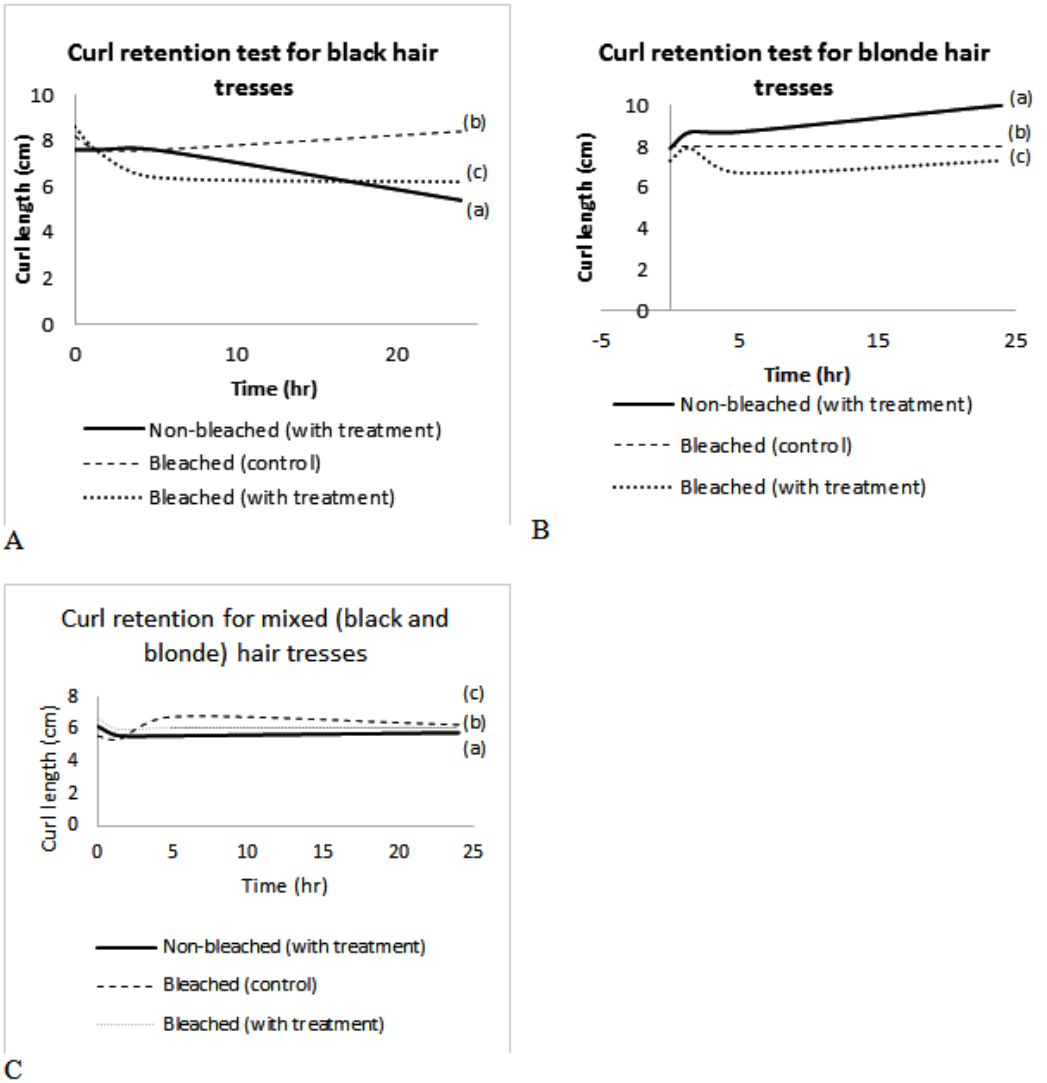

Fig. 3. Curl retention test: Length of black hair tresses at 0hr, 1.5hr, 5hr and 24hr after treatment with hair tonic (HT A formulation); (a): non-bleached hair with treatment (negative control); (b): bleached hair with no treatment (control); (c): bleached hair with treatment (positive control). A) Black hair, B) Blonde hair, C) Mixed (black and blonde) hair.

Table 6. Organoleptic properties of a hair tonic sample (HT A formulation) containing White Tea extract at different environmental conditions

Days	Organoleptic properties		Environmental condition Temperature Room temperature (25°C ± 2°C)	Light Exposure Drying Chamber (50°C ± 2°C)	Humidity With sunlight (25°C ± 2°C)	No sunlight (25°C ± 2°C)	Humidity Chamber (40°C ± 2°C; 75RH)
0	Colour		P. 120 Light golden yellow	P. 120 Light golden yellow	P. 120 Light golden yellow	P. 120 Light golden yellow	P. 120 Light golden yellow
	Surface appearance		C	C	C	C	C
	Clarity		C	C	C	C	C
	Texture		TW	TW	TW	TW	TW
	Odour	In the bottle	M	M	M	M	M
		On application	M and TS	M and TS	M and TS	M and TS	M and TS
	Intensity of odour	In the bottle	3	3	3	3	3
		On application	2	2	2	2	2
14	Colour		P. 121 Bright golden yellow	P. 128 Deep golden yellow orange tint	P. 122 Yellow with a slight yellow	P. 120 Light golden yellow	P. 121 Bright golden
	Surface appearance	C	C	C	C	C	
	Clarity	C	C	C	C	C	
	Texture	TW	TW	TW	TW	TW	
	Odour	In the bottle	M	M	M	M	M
		On application	M and TS	-	M and TS	M and TS	M and TS
	Intensity of odour	In the bottle	2	2	2	2	2
		On application	2	-	1	1	1
28	Colour		P-121 Bright golden yellow	P-115 Deep golden yellow slight orange tint	P-122 Yellow with a yellow	P-121 Bright golden	P-128 Deep golden yellow
	Surface appearance		Floating residues are present	C	C	C	C
	Clarity		C	C	C	C	C
	Texture		TW	TW	TW	TW	TW
	Odour	In the bottle	M	M	M	M	M
		On application	M and TS	TS	M and TS	M and TS	M and TS
	Intensity of odour	In the bottle	2	1	2	1	2
		On application	2		1		1

Table 4 displays the organoleptic properties of Hair Tonic A formulation stored at two different temperatures 25°C ± 2°C and 50°C ± 2°C), under light exposure and in an area with high humidity (40°C ± 2°C; 75RH) for 28 days (4 weeks). Abbreviations: P – Pantone code colour; C – Clear; TW – Thin and watery; M – Aromatic smell of Menthol; TS – Musty smell of Thermal Shield

Table 7. Organoleptic properties of a hair tonic sample (HT B formulation) containing Green Tea at different environmental conditions

Day	Organoleptic properties		Environmental condition				
			Temperature		Light Exposure		Humidity
			Room temperature (25°C +/- 2°C)	Drying Chamber (50°C +/- 2°C)	With sunlight (25°C +/- 2°C)	No sunlight (25°C +/- 2°C)	Humidity chamber (40°C +/- 2°C; 75RH)
0	Colour		P-120 Light golden yellow	P-120 Light golden yellow	P- 120 Light golden yellow	P-120 Light golden yellow	P-120 Light golden yellow
	Surface appearance		C	C	C	C	C
	Clarity		C	C	C	C	C
	Texture		TW	TW	TW	TW	TW
	Odour	In the bottle Upon application	M	M	M	M	M
	Intensity of odour	In the bottle	3	3	3	3	3
		On application	2	2	2	2	2
14	Colour		P-142 Mustard yellow	P-159 Dark amber brown	P-153 Dark amber brown	P-138 Amber brown	P-152 Orange-amber
	Surface appearance		C	C	C	C	C
	Clarity		C	C	C	C	C
	Texture		TW	TW	Less TW	TW	TW
	Odour	In the bottle	M	M	M	M	M
		On application	M	MS	MS	MS	MS
	Intensity of odour	In the bottle On application	3	3	3	3	3
28	Colour		P-137 Orange	P-166 Orange-red	P-3564C Orange-amber brown	P-137 Pale amber	P-152 Orange-amber
	Surface appearance		C	C	C	C	C
	Clarity		C	C	C	C	C
	Texture		TW	TW	TW	TW	TW
	Odour	In the bottle	MS	MS	MS	MS	M
		On application	MS				MS
	Intensity of odour	In the bottle On application	2	2	2	2	2 1

Table 5 displays the organoleptic properties of Hair Tonic B formulation stored at two different temperatures 25°C ± 2°C and 50°C ± 2°C), under light exposure and in an area with high humidity (40°C ± 2°C; 75RH) for 28 days. Abbreviations: P – Pantone code colour; C – Clear; TW – Thin and watery; M – Aromatic smell of Menthol; MS – Musty smell

Hair Weight

After 24 hours of treatment with the hair tonic, an increase in hair weight was observed in most bleached hair types, with the least significant change noted in mixed (black and blonde) hair tresses. For example, the weight of bleached black hair tresses increased from 28.28 g to 28.68 g, while the weight of bleached blonde hair tresses increased from 12.00 g to 12.15 g. The bleached mixed hair tresses exhibited varying results in terms of hair weight after 24 hours. Among the non-bleached hair types, only non-bleached black hair showed a decrease in weight after treatment compared to other non-bleached hair types. According to the two-way ANOVA test using Friedman's statistic, followed by Dunn's multiple comparison test, there was no significant difference in the hair weight between untreated and treated tresses after 24 hours ($p = 0.1667$; $p > 0.05$).

Based on Table 4, an increase in hair weight was observed over 24 hours in most bleached hair types, with the least significant change noted in mixed (black and blonde) hair tresses. Bleached mixed hair tresses showed inconsistent results, possibly due to measurement error. The higher porosity of bleached hair allows it to retain more product, reducing product loss from the hair shaft over time [35]. The reduced weight in non-bleached black hair may be due to fewer static forces between the hair strands after treatment. Dry hair can develop static forces that repel the strands, trapping air and contributing to hair weight. The hair tonic coats the surface, adding moisture and preventing static. As the product evaporates, static forces may return, affecting hair weight. Reapplication of the hair tonic may be needed after a few hours to maintain its effects [36].

Anti-frizz and anti-static analysis

After hair treatment, the combed hair length was longer in both the negative control (non-bleached with treatment) and positive control (bleached with treatment) groups compared to the control group (bleached with no treatment). Additionally, all non-bleached hair types displayed better manageability, with combed hair lengths being twice as long as those of bleached hair types after hair tonic application. For example, non-bleached black hair (negative control) was combed to a length of 21.1 cm, which is double that of the bleached hair (positive control). Two-way ANOVA, using Friedman and Dunn's multiple comparison tests, was conducted to assess the anti-frizz performance of the hair tonic across all hair types (Table 5). The treated hair tresses exhibited reduced static effects compared to untreated tresses, as evidenced by less frizz after dry combing (Figure 2).

In the anti-frizz analysis, all hair tresses were combed once using a fine-toothed comb until the comb encountered a tangle. Observations showed that both non-bleached and bleached hair types exhibited improved manageability after treatment, compared to the control group. Dry combing force is a useful indicator for evaluating a hair product's efficacy in detangling [10]. The application of the hair tonic smoothed the hair, making it tangle-free. Visual observations revealed that the control group showed the most frizz, characterized by flyaway or raised hairs. Static generated during combing caused positive charges to form in the hair, leading to strand repulsion. After hair tonic treatment, no frizz was observed in either the blonde or mixed (black and blonde) hair tresses. Dark-coloured Asian hair has more compact cuticle layers than Caucasian hair, and thin, fine hair with more cuticle surface area is more prone to static than thicker hair [37]. However, the findings indicated that black hair exhibited more static forces than lighter-colored hair. This suggests that, depending on hair volume, a larger amount of hair tonic may be required to achieve an anti-static effect. The standardized amount of hair tonic used was sufficient for both blonde and mixed hair to control flyaways effectively.

Figure 2. Results of sliding comb in strands before and after treatment with Hair Tonic A, followed by 10 combing strokes.

Curl retention test

As illustrated in Figure 3A, the non-bleached (normal) black hair tress showed curl retention for 5 hours before beginning to lose its curl after treatment with the hair tonic. In contrast, as seen in Figure 3B, the normal blonde hair tress did not exhibit the same effect, with the curl length increasing from 7.9 cm to 8.7 cm over 24 hours. Figure 3C shows that the normal mixed (black and blonde) hair tress experienced a reduction in curl length from 6.2 cm to 5.8 cm over 24

hours in the humidity chamber. Additionally, both bleached black and blonde hair showed reduced curl length after hair tonic application, indicating a curl retention effect. No significant change in curl length was observed in the bleached mixed (black and blonde) tresses over 24 hours. The control group (bleached hair with no treatment) in Figures 3A and 3B remained consistent in its curl length over 24 hours, with only a slight reduction in the first few hours. In Figure 3C, the control group demonstrated an increase in curl length 5 hours after exposure to humidity.

Both Hair Tonic A and Hair Tonic B demonstrated efficacy in improving hair thickness, anti-frizz properties, and curl retention in in vitro testing. However, Hair Tonic A consistently showed superior performance compared to Hair Tonic B across all efficacy parameters. Treatment with Hair Tonic A resulted in a more pronounced increase in hair thickness, particularly in bleached blonde hair, where thickness increased from 0.04 mm to 0.07 mm. Hair Tonic B, while effective, exhibited a less significant increase in hair thickness (from 0.04 mm to 0.06 mm in bleached hair). The higher efficacy of Hair Tonic A may be attributed to the higher concentration of catechins and polyphenols in white tea extract, which could promote better absorption and retention within the hair fibers.

Anti-Frizz Properties: Hair Tonic A demonstrated superior anti-frizz effects, as evidenced by longer combed hair lengths in treated hair tresses. For example, the combed hair length of black hair treated with Hair Tonic A reached 21.1 cm, compared to 11.8 cm for Hair Tonic B. Similarly, blonde hair treated with Hair Tonic A showed a combed length of 48.5 cm, significantly longer than Hair Tonic B-treated hair (29.2 cm). The higher viscosity of Hair Tonic A may have contributed to its better ability to coat and smooth hair fibers, thereby reducing frizz more effectively. Curl Retention: Hair Tonic A also showed better curl retention under humid conditions, maintaining curl structure for up to 24 hours in both bleached and non-bleached hair types. Hair Tonic B, on the other hand, exhibited reduced curl retention, particularly in bleached hair under high humidity. The superior curl retention of Hair Tonic A can be attributed to the presence of stable bioactive compounds in white tea extract, which may help preserve the structural integrity of hair fibers even under moisture-laden conditions.

The results from this study clearly demonstrate that Hair Tonic A exhibits superior stability and efficacy compared to Hair Tonic B. The white tea extract in Hair Tonic A contributed to its enhanced resilience under various environmental conditions, including elevated temperatures, high humidity, and light exposure. Additionally, Hair Tonic A outperformed Hair Tonic B in improving hair thickness, reducing frizz, and maintaining curl retention, especially in bleached and porous hair types. These findings highlight the importance of bioactive ingredient selection, with white tea extract offering greater protection and efficacy in hair tonic formulations compared to green tea extract.

In the curl retention test, the hair tresses were exposed to humid conditions. When hair is exposed to humidity, it tends to lose its structure as an increased number of hydrogen bonds form between keratin proteins due to excess moisture being absorbed into the hair. Keratin is held together by weak hydrogen bonds, which allow the hair to be styled. These bonds can be broken by water or moisture and reform as the hair dries [37]. Overall, the hair tonic positively improved the curls of all bleached hair types, as well as normal black and mixed (black and blonde) hair tresses. However, more product may be needed to maintain curls in non-bleached blonde hair tresses. The hair tonic was able to preserve curls throughout the day, thus fulfilling the "long-lasting curls" claim [38]. The curl retention effect of the hair tonic is linked to one of its main ingredients, FSS Kera Straightening & Curl. This ingredient contains the enzyme laccase, which facilitates cross-linkage formation between carboxylic acid groups in Hydrolyzed Keratin and amine groups in the hair cuticle, helping to retain the hair's shape. Additionally, FSS Kera Straightening & Curl provides protection against heat damage from hair styling.

Stability testing of Hair Tonic

Based on Table 6, the results show no significant difference in the organoleptic properties of the formulation stored at room temperature or under sunlight when placed in an amber-colored bottle. Since no UV light can penetrate the amber bottle, HT A is considered light and temperature

sensitive. There were no changes in the odor of Hair Tonic A samples on Days 14 and 28 across all conditions. However, based on Table 4, HT B exhibited color changes on Days 14 and 28 under all conditions, indicating that HT B is unstable under most conditions (Table 7). On Day 14, samples stored in all conditions, except at room temperature, developed a musty smell. By Day 28, the sample stored at room temperature also produced a musty odor upon application. Both HT A and HT B formulations are thin, watery, and clear.

Stability testing of Hair Tonic A and Hair Tonic B

The stability of both Hair Tonic A and Hair Tonic B was evaluated under multiple environmental conditions, including room temperature (25°C), elevated temperature (50°C), high humidity (75% RH at 40°C), and exposure to sunlight. Hair Tonic A (containing white tea extract) demonstrated superior stability under all tested conditions when compared to Hair Tonic B (containing green tea extract). Both formulations showed stable physicochemical properties at room temperature over the 28-day period, with minimal changes in their organoleptic characteristics. Hair Tonic B, however, exhibited a subtle color shift from light golden yellow to mustard yellow by Day 28, indicating early signs of instability, a change not observed in Hair Tonic A. When exposed to higher temperatures (50°C), Hair Tonic A maintained its physicochemical properties with no significant colour or odour changes. Hair Tonic B, however, showed notable instability by Day 14, with a shift in color from light golden yellow to dark amber brown and the development of a musty odour. This suggests that the bioactive compounds in green tea extract may degrade more rapidly under heat compared to white tea extract, which proved to be more heat stable. It is recommended to add a water-soluble fragrance to improve the product's scent. Overall, Hair Tonic A is organoleptically stable at room temperature and when stored in an amber bottle [39]. Under high humidity (75% RH at 40°C), Hair Tonic A displayed greater resilience, with only minor changes in organoleptic properties by Day 28. In contrast, Hair Tonic B exhibited significant instability, with a shift in colour and the development of a musty odour. These results indicate that the higher moisture resistance of Hair Tonic A may be attributed to the specific antioxidant and phenolic profile of white tea extract. Hair Tonic A also outperformed Hair Tonic B in terms of light stability. While Hair Tonic A retained its colour and clarity throughout the exposure period, Hair Tonic B showed substantial discoloration, changing from light golden yellow to orange-red by Day 28. Additionally, Hair Tonic B developed a musty odour after light exposure, suggesting a more rapid degradation of the active compounds in green tea extract. The humidity chamber samples also showed similar colour changes and developed a musty odour. Hair Tonic B is not stable in most environmental conditions, including room temperature, high temperature, and high humidity [40]. To improve its shelf life, adding preservatives with antimicrobial properties is recommended. Alternatives to parabens, such as p-anisic acid and levulinic acid, can be used to maintain stability and hair moisture [41].

CONCLUSION

The polyherbal hair tonic formulations developed in this study demonstrated promising results in terms of physicochemical stability and efficacy across different hair types. Hair Tonic A, containing white tea extract, showed superior stability, maintaining its physicochemical properties under varying environmental conditions compared to Hair Tonic B, which exhibited instability at elevated temperatures and under light exposure. Both formulations improved hair texture, thickness, and anti-frizz properties, with Hair Tonic A outperforming Hair Tonic B, particularly in curl retention and overall manageability. The formulations provided significant benefits in enhancing hair hydration, reducing frizz, and improving combability, particularly in bleached and porous hair types, which absorbed the formulations more effectively. The combination of multiple bioactive herbal extracts offers a multifaceted approach to hair care, functioning as a leave-in treatment capable of addressing a range of concerns such as split ends, heat protection, and scalp health without the use of harsh chemicals. FTIR analysis confirmed the chemical compatibility of the ingredients, supporting the

structural integrity of the formulations. These results suggest that polyherbal hair tonics, particularly Hair Tonic A, have strong potential for commercial adaptation as multifunctional hair care products. The incorporation of natural, bioactive ingredients aligns with the growing consumer demand for sustainable and eco-friendly cosmetics. Future research could explore further optimization of the formulations for enhanced shelf-life and the inclusion of additional bioactive compounds to broaden the scope of their therapeutic effects.

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This research does not involve any clinical trials

Authors' Contribution

Rajan Rajabalaya contributed to the conceptualization, methodology, writing of the original draft, and supervision. Nur Amalina Aliudin was responsible for data collection, analysis, and writing – review & editing. Sheba Rani David contributed to visualization, supervision, project administration, resources, and overall supervision

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