ORIGINAL RESEARCH PAPER

Dermatology

IN-VITRO STANDARDIZATION AND VALIDATION STUDY: INSTRUMENTAL AND EXPERT ASSESSMENT FOR EVALUATING EFFICACY OF HAIR AESTHETICS AND SENSORY ATTRIBUTES: SHINE, FRIZZ, SPLIT END REPAIR, TENSILE STRENGTH, AND MOISTURE CONTENT.

KEY WORDS: Frizziness, Split end repair, Hair Strength, Moisture Content, Hair Shine, Sensorial Assessment

Dr Nayan Patel M.B.B.S., Medical Director, Novobliss Research Private Limited. Office# A - 206, Shaligram Lakeview, Nr. Vaishnodevi Circle, Khoraj, Gandhinagar – 382 421, Gujarat, India.

Dr Maheshvari Patel* PhD Scholar, Pharmacology, Swaminarayan University, Kalol, Gandhinagar – 382 725, Gujarat, India, Director-Operations, Novobliss Research Private Limited. Office# A - 206, Shaligram Lakeview, Nr. Vaishnodevi Circle, Khoraj, Gandhinagar – 382 421, Gujarat, India.*Corresponding Author

Dr Apeksha Merja MD Dermatology, Sub-investigator, Novobliss Research Private Limited. Office# A - 206, Shaligram Lakeview, Nr. Vaishnodevi Circle, Khoraj, Gandhinagar – 382 421, India.

Jemini Pandya M. Pharm., Assistant Project Manager, Novobliss Research Private Limited. Office# A - 206, Shaligram Lakeview, Nr. Vaishnodevi Circle, Khoraj, Gandhinagar – 382421, Gujarat, India.

Nistha Jani M.Sc., Assistant Project Manager, Novobliss Research Private Limited. Office# A - 206, Shaligram Lakeview, Nr. Vaishnodevi Circle, Khoraj, Gandhinagar – 382421, Gujarat, India.

Dr Ravikumar R. Patel PhD, Dean & Principal, Shree Swaminarayan College Of Pharmacy, Swaminarayan University, Kalol, Gandhinagar, Gujarat, India.

ABSTRACT

Background: Hair quality assessment is crucial for evaluating the efficacy of hair care products. This study aimed to develop and validate In-Vitro methods for assessing efficacy of the various hair parameters using specialized techniques. **Methods:** Nine hair tresses were used per evaluation parameter, each hair tress standardized in weight, length, and color. Hair shine was measured with Glossymeter GL 200, frizz by Image-Pro software, split end repair via microscopic evaluations, tensile strength using TESTRONIX tensile tester, and porosity by the floating method. Hair sensorial attributes were assessed by an expert panel (6 panel experts) in a blinded fashion. Statistical analysis was conducted using SPSS (Version 29.0.1.0(171). **Results:** Significant improvements were observed in hair shine across all groups: the control group showed an increase ($p < 0.001$), the shampoo-treated group improved by 20.51% ($p < 0.0001$), and the shampoo followed by conditioner group by 44.34% ($p < 0.0001$). Hair frizziness was notably reduced: the control group by 1.73% ($p < 0.001$), the shampoo-treated group by 28.23% ($p = 0.0001$), and the shampoo followed by conditioner group by 31.28% ($p < 0.0001$). Hair split end repair metrics indicated reductions in distance (control: 5.76%, $p < 0.001$; shampoo: 26.12%, $p < 0.001$; shampoo + conditioner: 49.54%, $p < 0.001$) and angle between strands (control: 26.43%, $p < 0.0001$; shampoo: 36.49%, $p < 0.001$; shampoo + conditioner: 52.92%, $p < 0.001$). Sensorial parameters significantly improved in both treated groups compared to control, with the shampoo followed by conditioner group showing superior enhancement in frizz, smoothness, silkiness, softness, conditioning feel, moisturized feel, and combability. **Conclusions:** This study successfully developed and validated methods for In-Vitro efficacy assessment of hair parameters, including shine, frizz, split end repair, sensorial attributes, tensile strength, and porosity. The methods demonstrated consistent and reliable results, highlighting their potential as standardized approaches in evaluating hair care product efficacy performance under controlled conditions.

INTRODUCTION

Hair is a unique bio-composite primarily composed of proteins, constituting about 70% of its structure. These proteins are categorized into keratin and keratin-associated proteins (Gala, Muchhala, & Paspulate, Evaluation of sensory parameters and tensile strength on damaged hair after ten washes with Mintop shampoo: in vitro study, 2023). Keratin, rich in cysteine, forms an insoluble helicoidal protein complex (Chemical and physical damage affect the perceptions of hair attributes: A quantitative sensory assessment by a trained panel, 2020). The structure of hair includes two main components: the root and the shaft. The shaft itself comprises the cuticle, cortex, and, in thick hair, the medulla. The cuticle, the outermost layer, serves as a protective barrier against environmental factors. The cortex, containing melanin responsible for hair colour, provides tensile strength and elasticity (Gama & Baby, 2017). Melanin exists in two types: eumelanin (black-brown) and pheomelanin (red).

Healthy hair, enriched with approximately 80% alpha-keratin, exhibits qualities such as strength, flexibility, and durability (Jiang & Xu, 2023). It appears clean, soft, shiny, and bouncy (D'Souza P, 2015). However, hair damage results from the breakdown of keratin compositions and chemical bonds like iron bonds, disulphide bonds, and intermolecular hydrogen bonds, diminishing its mechanical properties (Qu, Guo, & Xu, 2022).

Various environmental factors and cosmetic treatments contribute to hair damage, including excessive heat, vigorous brushing, chemical treatments such as colouring and bleaching, and salon procedures (Jeong, Lee, Jeong, Kim, & Lee, 2010). Damaged hair is characterized by dullness, frizziness, dryness, and split ends, affecting its appearance and texture (Gala, Muchhala, & Paspulate, Evaluation of sensory parameters and tensile strength on damaged hair after ten washes with Mintop shampoo: in vitro study, 2023).

With increasing consumer concern for both aesthetic and health reasons, the efficacy of hair care products has become a significant focus. This *In-Vitro* study aims to validate methods for assessing parameters of hair care rinse-off

products. Specifically, it investigates these parameters using Indian black hair tresses measuring 12 inches in length, 1 inch in width, and weighing 5 grams. Assessments include hair shine, frizziness, split end repair, hair strength, hair porosity, and sensorial aspects using various instruments and dermatological evaluations.

METHODS

Study Design

This study employed various standardized methods tailored for assessing the effects of hair care products on different parameters. It was conducted in two phases: pre-wash evaluations and post-wash evaluations. The assessment of sensorial attributes was carried out in a randomized, double-blind manner, while parameters such as hair frizz, shine, tensile strength, moisture content (hair porosity), and split end repair were evaluated in an open-label format.

Ethics

In *In Vitro* laboratory testing using hair tresses, no human subjects are involved, eliminating the need for ethics committee approval and CTRI registration. Consequently, these approvals are not sought. However, the entire study was conducted in accordance with ICH-GCP guidelines and Good Laboratory Practice.

Test Product Usage

Two hair care rinse-off products were tested: Test Product A (Shampoo) and Test Product B (Shampoo followed by Conditioner). Approximately 3 mL of each test product was applied to each tress according to the randomization code, using 20 gentle strokes from top to bottom to ensure even distribution. The shampoo was left on the tresses for 3 minutes before rinsing with water. For the shampoo followed by conditioner regimen (Test Product B), approximately 5 mL of conditioner was applied after shampooing and left on the tresses for 5 minutes before rinsing with water. (Refer Table 1)

Table 1: Test Product Details

	Test Product A	Test Products B
Product Name	Hair Shampoo	Shampoo and Conditioner
Ingredient	Sodium Lauryl Sulfate Cocamidopropyl Betain, Water, Sodium Chloride, Glycol Distearate, Dimethicone, Panthenol, Fragrance	Water, Emollients, Cationic surfactants, Humectants, Glycerin, Propylene glycol, Proteins, Vitamins and Panthenol, Fatty Alcohols, Fragrance Thickeners and Emulsifiers, Acidifiers
Dosage Form	Liquid	Liquid
Route of Administration	Topical	Topical
Dosage	3 mL	5 mL
Type of Test Product	Rinse-Off Product	Rinse-Off Product

Pre-Treatment Phase

The hair tresses were washed with a 15% Sodium Lauryl Ethyl Sulfate (SLES) solution (approximately 1 mL per tress) to remove sebum and debris. After washing with SLES, the hair tresses were air-dried overnight at room temperature and then gently combed to detangle knots (Refer Figure 1).

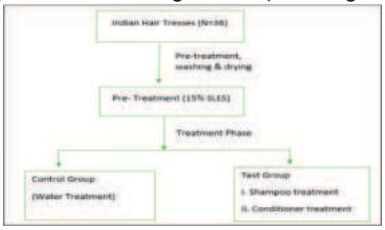

Figure 1: *Process flow*

Treatment Phase

Following the pre-treatment phase, baseline assessments were conducted. The appropriate amount of test product was applied to the hair tresses (refer table 1). Tresses in the control group were washed with water only (see Figure 2). While tresses in the test group were treated with the shampoo and shampoo + conditioner.

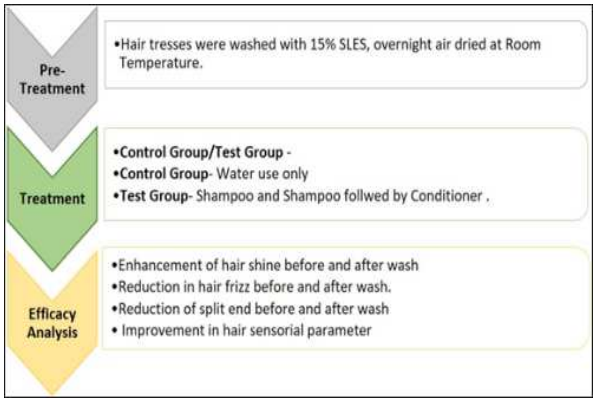

Figure 2: *Study Process*

Standardization

Standardization is the optimal approach to enhance compatibility, interoperability, safety, repeatability, and quality assurance of processes. Additionally, the standardization process itself can be standardized This standardization and validation study were conducted at NovoBliss Research in Mar 2024 demonstrating the processes and methods for testing the *In-Vitro* efficacy of hair care parameters, thereby ensuring compliance with the desired standards.

Training and Validation of Expert Panel Members for the Sensorial Assessment

Expert panellist training for hair sensorial assessment was conducted at NovoBliss Research before the study commenced annually. Six panellists were selected and trained to articulate sensations related to specific attributes: shine, silkiness, smoothness, softness, moisturized feel, combability (wet and dry hair), volume, frizz, and conditioning effect by the Dermatologist. The assessments were performed under controlled conditions of standard temperature (20-25°C) and humidity (30-70%), ensuring a double-blind approach with scoring on both 5-point and 10-point scales. Randomization protocols were implemented to mitigate bias.

Figure 3: *Hair Sensorial Assessment*

Scoring Scales

5-Points and 10 Points Scoring Scale

The hair sensorial parameter was assessed using both a 5-points and a 10-points scoring scale. On the 5-points scale, a score of 1 denotes "very poor" and a score of 5 denotes "very good." On the 10-points scale, a score of 1 represents "low/none/difficult" and a score of 10 represents "high/easy". (zakharenko, 2023)

Hair Shine Using Glossymeter GL 200

The Glossymeter GL 200(Courage + Khazaka) was utilized to measure hair shine. Each tress underwent pre-treatment with 1 mL of a 15% SLES solution followed by overnight drying at room temperature. Subsequently, the tresses were exposed to environmental pollutants for 48 hours, followed by baseline (pre-wash) assessments. The tresses were then divided into three groups: a control group, a group treated with shampoo only, and a group treated with shampoo followed by conditioner. Shine measurements were taken at the rear end of each tress both before and after treatment, providing a comprehensive assessment of product effects. (refer figure 3)

Figure 4: *Hair shine assessment by Glossymeter GL 200*

Hair Split End Repair Assessment Using Magnus Biological Microscope

This study involved an *In-Vitro* analysis of the effects of shampoo and conditioner on human hair tresses. A total of 9 tresses, each 12 inches in length and uniformly colored black (Indian origin), were used. The tresses were divided into three groups: control (n=3), shampoo treatment (n=3), and combined shampoo and conditioner treatment (n=3). Initially, the tresses were combed to remove knots and ensure uniformity. Five strands exhibiting split ends were then selected from each tress for microscopic examination (Magnus biological microscope, MX21iLED). High-resolution images were captured for each strand to facilitate comprehensive image analysis. For the treatment phase, 15 strands were rinsed with water only, 15 strands were treated with shampoo alone, and 15 strands received both shampoo and conditioner treatments. After treatment, the same microscopic examination and image analysis procedure were repeated to ensure consistency in strand orientation (Refer figure 5)

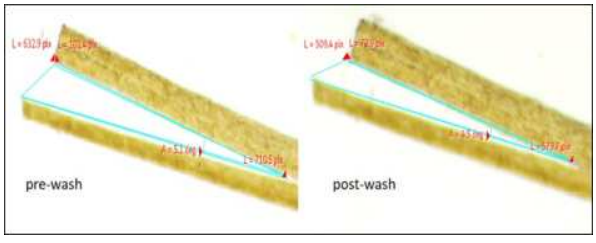

Figure 5: *Assessment of hair split end repair pre- and post-wash*

Hair Frizz Assessment Using Image-Pro

A total of 09 tresses with uniform characteristics (12 inches in height and Indian black color) were taken. Initially, hair tresses were combed to detangle the knots. High-resolution images were taken using a Nikon D3300 digital camera in JPEG format. Image analysis of each tress was performed by using Image-Pro® software (Version 10). Afterwards, Hair tresses were divided into three groups. 03 tresses for the control / water group, 03 tresses for the shampoo treated, and the remaining 03 tresses for the shampoo + conditioner treated. Tresses were washed with the respected products. After washing, hair tresses were dried at room temperature followed by combing to detangle the knots. Post-product usage assessment was performed. (Refer figure 4)

Figure 6: *Assessment of hair frizziness pre- and post-wash*

Hair Tensile Strength Assessment Using TESTRONIX

Hair strength was measured using a TESTRONIX tensile hair tester (Model No. TX TST (C), TESTRONIX), ensuring calibration and setup according to manufacturer guidelines. Clean, dry hair samples were cut to a standardized length of approximately 15 cm (6 inches), and each strand's diameter was measured with a micrometer to calculate cross-sectional area. Hair samples were securely mounted in the tester, one end in the upper clamp and the other in the lower clamp, ensuring straight and taut alignment. Testing commenced with gradual application of pulling force until each strand broke, recording the maximum force applied. Tensile strength was calculated using the formula: Tensile Strength = Force / Cross-sectional Area, providing a measure of hair strength in Mega Pascal (MPa).

Figure 7: *Tensile strength measurement using TESTRONIX*

Hair Porosity Assessment

Hair porosity was assessed using the floating test to understand moisture absorption and retention capabilities. Clean, dry hair strands were gently placed into the glass of water. Observations over 4 minutes determined porosity levels: strands that remained on the surface indicated low porosity, those sinking gradually to the middle indicated normal porosity, and those sinking quickly to the bottom indicated high porosity. This method provided insights into hair's response to moisture and chemical treatments. (https://caribbeansecretscosmetics.com/blogs/news/2-accurate-hair-porosity-tests-float-test-slipn-slide-test, 2022)

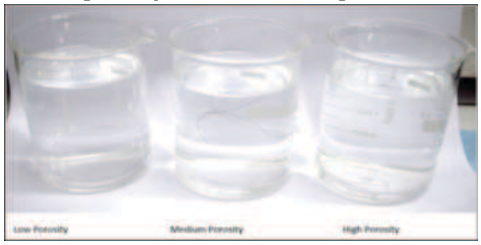

Figure 8: *Hair porosity assessment*

Statistical Analysis

Descriptive statistics were used to summarize continuous variables, including the count (N), mean, standard deviation (SD), median, minimum, and maximum values. Categorical variables were presented as frequencies and percentages, and graphical representations were employed when necessary.

Paired t-tests were conducted to analyse changes within groups across various time points, while independent sample t-tests were used to compare changes between different groups. Statistical analyses were performed using SPSS software (Version 29.0.1.0), with the significance level set at 5%.

RESULTS

A total of 36 hair tresses with Indian black colour were included in the study, each weighing 5 grams and measuring 12 inches in length and 1 cm in width, ensuring uniformity across all parameters.

Hair Shine Assessment

Quantitative assessment of hair shine using the Glossymeter GL200 revealed significant improvements post-application of test products. The control group showed a 0.48% increase in shine, whereas the shampoo-treated group and shampoo followed by conditioner group exhibited improvements of 20.51% and 44.34%, respectively (p-value <0.0001). Both treated groups demonstrated statistically significant enhancement in shine compared to the control group.

Hair Frizziness Assessment

Using Image-Pro software, reductions in hair frizziness were measured post-treatment. The control group saw a 1.74% reduction in frizziness area, while the shampoo-treated and shampoo followed by conditioner-treated groups showed reductions of 28.23% and 31.28%, respectively (p-value <0.0001). (Refer table 2)

Hair Split end repair

Analysis of split ends using Image-Pro software indicated significant reductions in distance and angle in all treated groups compared to the control. The control group showed reductions of 24.19% and 68.57%, the shampoo-treated group showed 69.66% and 66.67%, and the shampoo followed by conditioner-treated group showed 74.79% and 92.36%, respectively. All these results were statistically significant with p-values <0.01. (Refer table 2)

Table 2: Change in Hair Frizziness (px^2) and Split end repair

Change in Hair Frizziness (Image-Pro software)				
	Pre-Wash (x□ ± SD) (N=3)		Post wash (x□ ± SD) (N=3)	
Control	29.72 ± 2.82		29.20 ± 2.89	
Shampoo Treated	34.18 ± 2.33		24.53 ± 2.28	
Shampoo + Conditioner	31.17 ± 4.23		21.42 ± 4.16	
Change in Split End Repair (Image-Pro software)				
	Distance between two hair strands (N=15)		Angle between two hair strands (N=15)	
	Pre-Wash (x□ ± SD)	Post wash (x□ ± SD)	Pre-Wash (x□ ± SD)	Post wash (x□ ± SD)
Control	134.97 ± 67.79	128.93 ± 66.92	7.98 ± 3.39	6.04 ± 2.98
Shampoo Treated	198.91 ± 144.95	155.06 ± 134.66	9.93 ± 7.08	5.93 ± 4.19
Shampoo + Conditioner	215.02 ± 110.79	98.51 ± 45.35	8.58 ± 4.20	3.69 ± 1.42

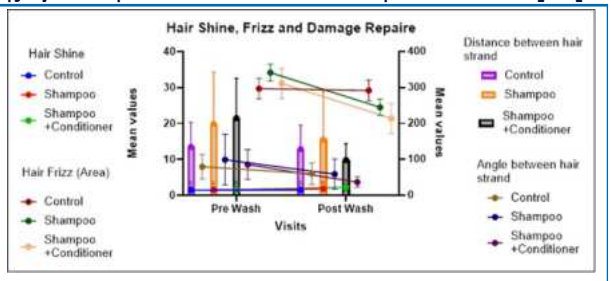

Figure 9: *Changes in hair shine, hair frizz and damage repair*

Hair Sensorial Parameters (5-points scoring scale)

Sensorial assessment was conducted in a randomized, double-blind manner. The control group did not show significant changes in sensory parameters except for silkiness, moisturized feel, and residue. In contrast, both the shampoo-treated and shampoo followed by conditioner-treated groups demonstrated significant improvements in all assessed sensorial parameters (p-value <0.0001). Post-application of the test products, both groups showed substantial improvements in wet feel, wet hair combability, force to comb, dry hair combability, force to comb dry hair, softness, smoothness, silkiness, moisturized feel, shine, bounce, volume, residue, conditioned feel, frizziness, healthy appearance, and nourished feel. (refer table 3) The shampoo followed by conditioner group exhibited higher improvements across all parameters compared to the control and shampoo-only groups. (refer table 4)

Table 3: Change in sensorial parameters within treatment 5-point scoring scale

Parameter	Sample	Pre-Wash (N=18)	Post Wash (N=18)	P-value
Hair Force to comb	Control	2.00 (0.00)	2.00 (0.00)	0.363
	Shampoo	2.33.00(0.01)	4.00 (0.00)	<0.001
	Shampoo + conditioner	2.28 (0.14)	5.00 (0.00)	<0.001
Hair Comb-ability	Control	2.00 (0.00)	2.00 (0.00)	0.363
	Shampoo	1.67 (0.00)	4.00 (0.00)	<0.001
	Shampoo + conditioner	1.94 (0.14)	5.00 (0.00)	<0.001
Hair Condi-tioned feel	Control	1.33.00(0.01)	1.33 (0.01)	NA
	Shampoo	1.00 (0.00)	3.67 (0.00)	<0.001
	Shampoo + conditioner	1.61 (0.14)	4.67 (0.00)	<0.001
Hair Moisturized feel	Control	1.33 (0.01)	1.67 (0.00)	<0.001
	Shampoo	1.00 (0.00)	3.00 (0.00)	<0.001
	Shampoo + conditioner	1.72 (0.14)	4.00 (0.00)	<0.001
Hair Frizziness	Control	2.00 (0.00)	2.00 (0.00)	NA
	Shampoo	2.33 (0.00)	4.00 (0.00)	<0.001
	Shampoo + conditioner	2.33 (0.01)	4.67 (0.00)	<0.001
Hair Shine / Gloss	Control	1.67 (0.00)	1.67 (0.00)	NA
	Shampoo	2.00 (0.00)	3.67 (0.00)	<0.001
	Shampoo + conditioner	2.00 (0.00)	4.67 (0.00)	<0.001
Hair Silkiness	Control	2.67 (0.00)	1.67 (0.00)	<0.001
	Shampoo	2.00 (0.00)	4.00 (0.00)	<0.001
	Shampoo + conditioner	2.33 (0.00)	4.67 (0.00)	<0.001
Hair Smoothness	Control	1.67 (0.00)	1.72.00(0.14)	0.363
	Shampoo	1.94 (0.14)	3.98 (0.04)	<0.001
	Shampoo + conditioner	2.06 (0.14)	4.67 (0.00)	<0.001
Hair Softness	Control	2.00 (0.00)	2.06 (0.14)	0.363
	Shampoo	2.33 (0.01)	3.72.00(0.14)	<0.001
	Shampoo + conditioner	2.06 (0.14)	4.67 (0.00)	<0.001

Hair Bounce	Control	1.00 (0.00)	1.00 (0.00)	0.363
	Shampoo	1.00 (0.00)	3.33.00(0.01)	<0.001
	Shampoo + conditioner	1.61(0.14)	3.67 (0.00)	<0.001
Hair Volume	Control	1.67 (0.00)	1.78 (0.17)	0.174
	Shampoo	2.00 (0.00)	3.00 (0.00)	<0.001
	Shampoo + conditioner	2.00 (0.00)	4.00 (0.00)	<0.001
Hair Healthy Appea-rance	Control	1.67 (0.00)	1.67 (0.00)	NA
	Shampoo	1.67 (0.00)	3.67 (0.00)	<0.001
	Shampoo + conditioner	2.28 (0.14)	4.33.00 (0.01)	<0.001
Hair Nourished Appearance	Control	1.00 (0.00)	1.00 (0.00)	0.363
	Shampoo	1.67 (0.00)	3.00 (0.00)	<0.001
	Shampoo + conditioner	1.67 (0.00)	4.00 (0.00)	<0.001
Residue	Control	2.33 (0.00)	1.89 (0.17)	0.001
	Shampoo	2.00 (0.00)	3.33.00(0.01)	<0.001
	Shampoo + conditioner	2.33 (0.01)	4.00 (0.00)	<0.001

Table 4: Change in sensorial parameters between treatment 5-point scoring scale

t Test					
Parameter	Control (N=18) [Mean (SD)]	Shampoo (N=18) [Mean (SD)]	p-value (Shampoo vs Control)	Shampoo + Conditioner (N=18)	p-value (Shampoo + Conditioner vs Control)
During Post Wash					
Wet Feel of Hair	2.00(0.84)	4.00(0.00)	<0.0001	4.33(0.49)	<0.0001
Force to Comb Wet Hair	2.00(0.84)	3.67(0.49)	<0.0001	5.00(0.00)	<0.0001
Wet Hair Combability	2.00(0.84)	4.00(0.00)	<0.0001	5.00(0.00)	<0.0001
After Post Wash (Change from Baseline)					
Dry Hair combability	0.00(0.24)	2.33(0.59)	<0.0001	3.00(0.91)	<0.0001
Force to Comb Dry Hair	0.00(0.00)	2.56(0.51)	<0.0001	2.61(0.61)	<0.0001
Softness	0.11(0.32)	1.28(0.57)	<0.0001	2.61(0.50)	<0.0001
Smoothness	0.00(0.97)	1.89(0.47)	<0.0001	2.56(0.62)	<0.0001
Silkiness	1.00(0.84)	1.89(0.32)	<0.0001	2.28(0.46)	<0.0001
Moisturized Feel	0.28(0.57)	1.94(0.24)	<0.0001	2.22(0.55)	<0.0001
Shine/ Gloss	0.06(0.24)	1.61(0.50)	<0.0001	2.61(0.50)	<0.0001
Bounce	0.06(0.24)	2.28(0.46)	<0.0001	2.00(0.84)	<0.0001
Volume	0.11(0.32)	0.94(0.24)	<0.0001	2.00(0.00)	<0.0001
Residue	0.50(0.51)	1.22(0.55)	<0.0001	1.61(0.50)	<0.0001
Conditioned Feel	0.00(0.00)	2.61(0.61)	<0.0001	3.06(0.87)	<0.0001
Frizziness	0.06(0.24)	1.67(0.49)	<0.0001	2.28(0.57)	<0.0001
Healthy Appearance	0.06(0.24)	1.94(0.24)	<0.0001	2.00(0.34)	<0.0001
Nourished Appearance	0.06(0.24)	1.33(0.49)	<0.0001	2.28(0.46)	<0.0001

Hair Sensorial Parameters (10-points scoring scale)

On the 10-point scoring scale for sensorial parameters assessment, the control group showed no significant changes except for improvements in softness, smoothness, residue, and moisturized feel.

In contrast, significant improvements were observed across all parameters in both the shampoo-treated and shampoo followed by conditioner-treated groups post-application of the test products (p-value <0.0001). (refer table 5)

The shampoo followed by conditioner group showed greater enhancement in all parameters compared to the control and shampoo-only groups. (refer table 6)

Table 5: Change in sensorial parameters within treatment 10-point scoring scale

Paired t Test				
Parameter	Sample	Pre-Wash (N=18) [Mean (SD)]	Post Wash (N=18) [Mean (SD)]	P- Value
Dry Hair Combability	Control	3.67 (1.28)	3.67 (0.97)	1
	Shampoo	3 (0)	8 (0)	<0.001
	Shampoo + Conditioner	3.67 (0.97)	9.33 (0.49)	<0.001
Force to Comb	Control	3.28 (1.07)	3 (0.84)	0.056
	Shampoo	3.33 (0.49)	7.67 (0.49)	<0.001
	Shampoo + Conditioner	3.67 (0.49)	9 (0)	<0.001
Frizziness	Control	3.56 (0.51)	3.33 (0.49)	0.163
	Shampoo	3.67 (0.49)	7.67 (0.49)	<0.001
	Shampoo + Conditioner	4 (0)	9 (0)	<0.001
Conditioned Feel	Control	2.67 (0.49)	2.67 (0.49)	-
	Shampoo	2 (0)	7 (0)	<0.001
	Shampoo + Conditioner	3 (0.84)	9 (0)	<0.001
Moisturized Feel	Control	2.33 (0.49)	3 (0.84)	<0.001
	Shampoo	2 (0)	6.33 (1.94)	<0.001
	Shampoo + Conditioner	2.94 (0.8)	9 (0.84)	<0.001
Shine/ Gloss	Control	3.22 (0.94)	3.33 (0.97)	0.163
	Shampoo	3.67 (0.49)	7.67 (0.49)	<0.001
	Shampoo + Conditioner	3.28 (0.46)	9 (0)	<0.001
Silkiness	Control	3.67 (0.49)	3.39 (0.5)	0.056
	Shampoo	3 (0)	8 (0)	<0.001
	Shampoo + Conditioner	3.28 (0.46)	9 (0)	<0.001
Smoothness	Control	3.33 (0.49)	2.94 (0.8)	0.004
	Shampoo	4 (0.84)	7.33 (0.49)	<0.001
	Shampoo + Conditioner	3.33 (0.49)	8.72 (0.96)	<0.001

Softness	Control	3.33 (0.49)	3 (0.84)	0.01
	Shampoo	4 (0.84)	7 (0)	<0.001
	Shampoo + Conditioner	3.33 (0.49)	8.72 (0.96)	<0.001
Bounce	Control	2.22 (0.65)	2 (0)	0.163
	Shampoo	2 (0)	7 (0)	<0.001
	Shampoo + Conditioner	3 (0.84)	8 (0)	<0.001
Volume	Control	2 (0)	2 (0)	-
	Shampoo	2 (0)	7 (0.84)	<0.001
	Shampoo + Conditioner	2.67 (0.49)	8 (0)	<0.001
Healthy Appearance	Control	2.67 (0.49)	2.67 (0.49)	-
	Shampoo	2.67 (0.49)	7 (0)	<0.001
	Shampoo + Conditioner	3 (0)	8.67 (0.49)	<0.001
Nouris hed Appearance	Control	2.33 (0.49)	2.33 (0.49)	-
	Shampoo	2 (0)	6.67 (0.49)	<0.001
	Shampoo + Conditioner	2.67 (0.49)	8.67 (0.49)	<0.001
Residue	Control	4 (1.19)	3.33 (0.49)	0.01
	Shampoo	4 (0)	6.67 (0.49)	<0.001
	Shampoo + Conditioner	4.33 (0.49)	8.33 (0.49)	<0.001

Table 6: Change in sensorial parameters between treatment 10-point scoring scale

t Test					
Parameter	Control (N=18) [Mean (SD)]	Shampoo (N= 18) [Mean (SD)]	p-value (Control vs Shampoo)	Shampoo + Conditioner (N= 18)	p-value (Control vs Shampoo + conditioner)
During Post Wash					
Wet Feel of Hair	3.33(1.28)	7.00(0.00)	<0.0001	8.00(0.00)	<0.0001
Force to Comb Wet Hair	3.67(1.75)	8.00(0.00)	<0.0001	9.00(0.00)	<0.0001
Wet Hair Combability	3.67(1.75)	7.33(0.49)	<0.0001	8.67(0.49	<0.0001
After Post Wash (Change from Baseline)					
Hiar combability	-0.17 (0.79)	5.22(0.55)	<0.0001	5.67(1.33)	<0.0001
force to comb	-0.22 (0.65)	4.22(0.88)	<0.0001	5.44(0.51)	<0.0001
softness	-0.28 (0.57	3.00(0.91)	<0.0001	5.39(1.29)	<0.0001
smoothness	-0.33 (0.49)	3.28(0.57)	<0.0001	5.44(1.29)	<0.0001
silkiness	-0.33 (0.59)	4.89(0.32)	<0.0001	5.67(0.49)	<0.0001
Moisturized Feel	0.50 (0.71)	4.61(1.42)	<0.0001	6.00(0.84)	<0.0001
Shine/ Gloss	0.06 (0.42)	4.22(0.73)	<0.0001	5.72(0.57)	<0.0001
Bounce	-0.28 (0.67)	5.00(0.34)	<0.0001	4.89(0.83)	<0.0001
Volume	0.00 (0.00)	5.00(0.77)	<0.0001	5.33(0.49)	<0.0001
Residue	-0.61(0.98)	2.78(0.55)	<0.0001	4.00(0.84)	<0.0001
Conditioned Feel	0.00 (0.00)	5.00(0.34)	<0.0001	6.00(0.77)	<0.0001
Frizziness	-0.22 (0.65)	4.00(0.77)	<0.0001	5.00(0.00)	<0.0001
Healthy Appearance	0.06 (0.24)	4.33(0.49)	<0.0001	5.56(0.62)	<0.0001
Nourished Appearance	0.00 (0.00)	4.67(0.49)	<0.0001	6.00(0.84)	<0.0001

Hair Tensile Strength

Tensile strength assessment indicated normal range values for all groups both before and after application of the test products. The shampoo followed by conditioner group demonstrated better hair strength compared to the control and shampoo-only groups. (refer table 7)

Table 7: Change in tensile strength measurement

Change in Hair Tensile Strength (MPa)		
	Pre-Wash (x̄ ± SD)	Post wash (x̄ ± SD)
Control	227.00± 101.46	239.71 ± 109.71
Shampoo Treated	226.52 ± 97.20	118.93 ± 105.28
Shampoo + Conditioner	255.64 ± 96.57	275.47 ± 109.69

DISCUSSION

This study aimed to comprehensively evaluate various parameters using different methodologies. Methods included hair shine assessment using the Glossymeter GL 200, frizz assessment via Image-Pro software, hair split end repair assessment through microscopic evaluations and Image-Pro software, sensorial parameter evaluation by expert panel, hair tensile strength testing with the TESTRONIX tensile tester, and hair porosity assessment using the floating method, all of which were validated for reliability. The results demonstrate distinct effects of the test products—shampoo, shampoo followed by conditioner, and the control group—across multiple parameters including hair shine, split end repair, frizziness, porosity, tensile strength, and sensorial qualities. Both the shampoo-treated and shampoo followed by conditioner-treated groups showed significant improvements in hair shine compared to the baseline and control group. Particularly, the shampoo followed by conditioner group exhibited more pronounced enhancements in hair shine, underscoring the superior efficacy of the combined treatment in hair care.

UV radiation is a primary cause of hair damage, leading to dryness, roughness, color change, dullness, and stiffness upon sun exposure. Compared to the baseline and control group, significant improvement in hair split end repair was observed in the shampoo followed by conditioner-treated group. Conditioners containing positively charged cationic compounds help deposit on negatively charged hair fibers, reducing friction, increasing light reflectance, and enhancing shine, smoothness, and color (D'Souza & Rathi, 2015).

Assessment of hair frizz using image analysis revealed reductions in both the shampoo-treated and shampoo followed by conditioner-treated groups compared to baseline and control. The latter group showed a more substantial decrease in frizz, attributed to the hydrating and smoothing properties of conditioners, which penetrate the hair shaft, restore moisture, and reduce frizziness and dryness (Dhawan, 2022).

Hair strength was assessed using a tensile hair tester. The

control and shampoo-treated groups exhibited lower resistance to breakage, while the conditioner-treated group required slightly higher force to break the hair, indicating improved hair strength. Hair porosity was evaluated using the float test. Various methods such as the strand test and spray test are commonly used for porosity assessment (CleverCurl, 2022). In our study, the floating method indicated no significant changes in hair porosity after using the rinse-off product.

Our study also evaluated hair sensorial parameters—including shine, smoothness, silkiness, dryness, combability, wet feel, conditioning effect, and moisturized feel—through a panel of six experts. Results showed that the group treated with shampoo followed by conditioner demonstrated significant improvements in these parameters compared to both the control and shampoo-only groups. Chemical treatments such as dyeing and straightening can compromise the mechanical and structural integrity of hair fibers, making them susceptible to damage. This underscores the growing demand for effective hair care products that can restore and maintain hair health. Shampoos are essential for removing sebum, debris, environmental pollutants, sweat, and applied hair care products from the scalp and hair. However, they can sometimes leave hair frizzy, dry, and difficult to manage. Conditioners play a crucial role in softening, smoothing, adding shine, increasing volume, improving manageability, and maintaining hairstyles (Draelos, 2013).

Significant improvements were observed across multiple parameters. Compared to the control group, there was a marked reduction in hair frizz, effective repair of split ends, and noticeable enhancement in both hair shine and sensorial qualities. Furthermore, the combination treatment of shampoo followed by conditioner yielded superior results compared to using shampoo alone, highlighting the synergistic benefits of this approach in hair care.

However, this study has limitations to consider. Firstly, this study was conducted in an *In-Vitro* setting, which may not fully replicate real-world conditions. Additionally, this study focused exclusively on Indian black hair tresses, limiting the generalizability of the assessment techniques employed to other hair types and ethnicities.

CONCLUSION

This study successfully developed and validated methods for the *In-Vitro* assessment of key hair parameters, including shine, frizz, split end repair, sensorial attributes, tensile strength, and porosity. The methods demonstrated consistent and reliable results, highlighting their potential as standardized approaches in evaluating the performance of hair care products under controlled laboratory conditions. The study effectively determined the significance in changes observed across multiple parameters like reduction in hair frizziness, repaired hair damage, enhanced hair shine, and improved hair sensorial attributes. These results underscore the importance of robust assessment techniques in advancing our understanding and evaluation of hair care innovations.

Moving forward, these validated methods pave the way for more precise and objective evaluations of new hair care formulations. By standardizing these assessment protocols, researchers and industry professionals can better predict and enhance the performance of hair care products, ultimately benefiting consumers with more effective solutions for maintaining and improving hair health.

Declarations

Funding: NovoBliss Research Private Limited

Disclosure: Authors report no conflict of interest.

Ethical approval: Not Applicable.

Abbrevations

SLES:Sodium Lauryl Ethyl Sulphate

Acknowledgements

The authors would like to express their sincere gratitude to NovoBliss Research Private Limited and their dedicated teams for their collaborative support throughout this study. Special thanks are extended to Dr Arnav Purani (Medical Writer) and study team for their diligent oversight of the *In-Vitro* study, the statistical team for their expertise in data analysis, and the scientific writing team for their invaluable assistance in manuscript preparation. A heartfelt acknowledgment is also due to the study team for their comprehensive management of the study.

REFERENCES

1. Chemical and physical damage affect the perceptions of hair attributes: A quantitative sensory assessment by a trained panel. (2020). *journal of sensory studies*. doi:doi.org/10.1111/joss.12621
2. CleverCurl. (2022, july 21). *HOW TO DETERMINE HAIR POROSITY AND WHAT IT MEANS FOR YOUR HAIR*. (https://www.clevercurl.com.au/how-to-determine-hair-porosity-and-what-it-means-for-your-hair)
3. Dhawan, S. (2022, May 28). *https://www.reequil.com/blogs/care-tips/hair-conditioners*. (Re'equil)
4. Draelos, Z. D. (2013). Shampoos, Conditioners, and Camouflage Techniques. *Dermatologic Clinics, 13*(1), 173-178. doi:10.4236/jcdsa.2013.33A1006
5. D'Souza, P., & Rathi, S. K. (2015). Shampoo and Conditioners: What a Dermatologist Should Know? *Indian journal of dermatology, 3*(60), 248-254. doi:10.1055/s-0041-1739241
6. Gala, M. Y., Muchhala, S., & Paspulate, A. (2023). Evaluation of sensory parameters and tensile strength on damaged hair after ten washes with Mintop shampoo: in vitro study. *International Journal of Research in Dermatology*. doi:10.18203/issn.2455-4529.IntJResDermatol20233178
7. Gama, R. M., & Baby, A. R. (2017). In Vitro Methodologies to Evaluate the Effects of Hair Care Products on Hair Fiber. *Cosmetics*. doi:10.3390/cosmetics4010002
8. *https://caribbeansecretscosmetics.com/blogs/news/2-accurate-hair-porosity-tests-float-test-slipn-slide-test*. (2022, April 1). Retrieved from Caribbean Secrets.
9. Jeong, M., Lee, C., Jeong, W., Kim, S., & Lee, K. (2010). Significant damage of the skin and hair following hair bleaching. *journal of dermatology, 37*(10). doi:10.1111/j.1346-8138.2010.00916.x
10. Jiang, Y., & Xu, Z. (2023). Comparative study of instrumental measurement and sensory evaluation methods for the repairing effect of mildly damaged hair bundles. *skin reserach and technology*. doi:10.1111/srt.13394
11. Qu, W., Guo, X., & Xu, G. (2022). Improving the Mechanical Properties of Damaged Hair Using Low-Molecular Weight Hyaluronate. *Natural Products and Biomolecules for Cosmetics Applications*. doi:10.3390/ molecules27227701
12. Stricane, C., M, D. R., & Sayer. (2013). Establishing Hair Care Sensory Testing Capabilities. *Cosmetic Science Technology*. Retrieved from https://www.cosmeticsciencetechnology.com/
13. zakharenko, a. (2023, May 8). *aidaform*. Retrieved from https://aidaform.com/blog/likert-scale-definition-examples.html