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Protective Effects of Neeli Bhringraj Oil Against Hair Breakage and Strengthening Damaged Hair Fibers: Scientific Evidence from an *In-Vitro* Fatigue Test Mode

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ABSTRACT

Hair fall due to breakage is a widespread concern influenced by multiple intrinsic and extrinsic factors such as heat styling, chemical treatments, pollution, and UV exposure. Herbal oils, particularly those used in Ayurvedic practice, have long been valued for their ability to protect and nourish hair. This study aimed to evaluate the efficacy of Neeli Bhringraj oil, a traditional Ayurvedic formulation, in reducing hair breakage and improving tensile strength using *in-vitro* fatigue test model. **Methods: Standardised** human hair swatches were deliberately damaged through surfactant washes, heat, UV, and pollution exposure. Damaged swatches were randomized and treated for 12 cycles with either Neeli Bhringraj oil (test group) and another with control. After treatment, swatches underwent wet fatigue testing using the TESTRONIX Tensile Strength Tester, where broken fibers were collected and analyzed. Statistical significance was assessed using paired two-tailed t-tests ($p < 0.05$). **Results: The Neeli Bhringraj oil group showed significantly fewer broken fibres** (11.67 ± 4.46) compared to the control group (21.67 ± 9.69, $p < 0.01$). This corresponded to a 4.19-fold improvement or 76.11% reduction in breakage. Fragment analysis further revealed fewer short-length fibers (<6.25 cm) in the test group, indicating stronger tensile resilience. **Conclusion: This study confirms that Neeli Bhringraj oil (Neela Bhringadi) provides substantial protection against hair breakage and effectively strengthens damaged hair fibres**. By forming a protective layer and nourishing the hair shaft, the oil minimizes damage from environmental and mechanical stressors. These findings validate the traditional use of Neeli Bhringraj oil and support its potential as an evidence-based intervention for reducing hair fall and improving overall hair health.

Key Words:

Keywords: Neeli Bhringraj, Herbal hair oil, Hair fall, Hair strengthening, Fatigue test, Efficacy, Hair Damage Repair.

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1. INTRODUCTION

Hair loss and breakage are among the most common cosmetic concerns globally, arising from intrinsic factors such as genetics and hormonal imbalance, as well as extrinsic stressors like pollution, UV radiation, frequent heat styling, and chemical-based hair care products [1]. These factors disrupt the structural integrity of hair, reducing tensile strength, increasing roughness, and causing excessive fall [2]. Regular oiling has long been advocated in traditional medicine and is increasingly validated by scientific research for its ability to nourish hair, improve strength, and protect against external damage [3].

Neeli Bhringraj oil, a well-recognized formulation in Ayurvedic medicine, has been extensively used to promote hair growth and maintain hair health [4]. Traditionally referred to as the "king of hair oils," it is known to strengthen hair shafts, reduce breakage, prevent premature hair fall, and support overall scalp vitality. Experimental studies have reported its role in enhancing hair follicle activity, prolonging the anagen phase of the hair cycle, and protecting against oxidative stress and environmental aggressors. These properties make Neeli Bhringraj oil a promising intervention for managing hair fall and improving hair resilience [5].

Despite its traditional use, there is limited systematic in vitro evaluation of Neeli Bhringraj oil using standardized methodologies. *In-vitro* fatigue testing of damaged hair swatches provides a reproducible and objective means to assess product efficacy under controlled conditions.

The present investigation was undertaken to evaluate the efficacy of Neeli Bhringraj oil in reducing hair fall due to breakage, enhancing tensile strength, and providing nourishment to damaged hair swatches under controlled *in vitro* conditions. The study compared the effects of Neeli Bhringraj oil with those of an appropriate vehicle control using a standardized fatigue test model.

1. Methodology

Test Product

The test formulation evaluated in this study was **Neeli Bhringraj oil**, a traditional Ayurvedic herbal preparation used for promoting hair health. The oil was enriched with *Indigofera tinctoria* (Neeli) and *Eclipta alba* (Bhringraj) extracts in a suitable carrier base. It is traditionally reported to nourish the scalp, strengthen hair shafts, and reduce hair fall associated with breakage.

For the control group, Coconut oil (*Cocos nucifera*) was used as the vehicle comparator, identified as Batch No. CO-201, manufactured in October 2024, with an expiry date of September 30, 2027.

For the present *in vitro* evaluation, the test oil was manufactured under controlled laboratory conditions following standardized extraction and blending procedures. The test batch was identified as Batch No. NBO-101, manufactured in October 2024, with an expiry date of September 30, 2027.

Botanical Name	Common Name
Hibiscus Rosa (Leaves)	Hibiscus Rosa (Leaves) Powder
Cuscuta Reflexa Roxb	Amarbel Powder
Nardostachys Jatamansi	Jatamansi Root Powder
Vitis Vinifera	Grape Seed Oil
Lawsonia Inermis	Henna Leaf Powder
Salvia Rosmarinus	Rosemary Leaves Powder
Centella Asiatica	Gotu Kala Powder (Whole Plant)
Azadirachta Indica	Neem Leaves
Trigonella Foenum	Fenugreek Seed
Murraya Koenigii	Curry Leaves
Phyllanthus Emblica	Amla Fruits
Cocos Nucifera	Coconut Oil
Ricinus Communis	Castor Oil

Study Design

This was an *in vitro*, randomized, double-blind, controlled study designed to evaluate the protective effect of Neeli Bhringraj oil on damaged hair swatches. The study compared the efficacy of the test oil with an appropriate vehicle control using a standardized fatigue test model. Randomization was applied to allocate hair swatches into treatment and control groups, and both the treatment administrator and outcome assessor were blinded to the group identity to minimize bias.

Damage Creation

To mimic real-life environmental and cosmetic stressors, standardized hair swatches were subjected to the following controlled damage protocols:

- **Surfactant Damage:** 12 wash cycles with 0.2 g/gram of hair or 14% of sodium lauryl ether sulfate (SLES).
- **Heat Damage:** 100 sweeps with a thermal straightening iron at 220°C, each sweep lasting 20 seconds.
- **UV Exposure:** 30 minutes of continuous UV exposure.
- **Pollution Exposure:** 30 minutes of exposure to automotive exhaust (145.45 cc, 6.6 kW at 5000 rpm for 20 minutes).

Treatment Groups

- **Test Group:** Damaged hair swatches were treated with Neeli Bhringraj oil, left for 2 hours, washed with non-conditioning shampoo, and dried using a hood dryer. This treatment cycle was repeated 12 times.
- **Control Group:** Damaged hair swatches were treated with **Coconut oil (vehicle control)**, left for 2 hours, washed with non-conditioning shampoo, and dried using a hood dryer. This treatment cycle was also repeated 12 times.

Figure 1: SLES Wash

Figure 2: Thermal Damage

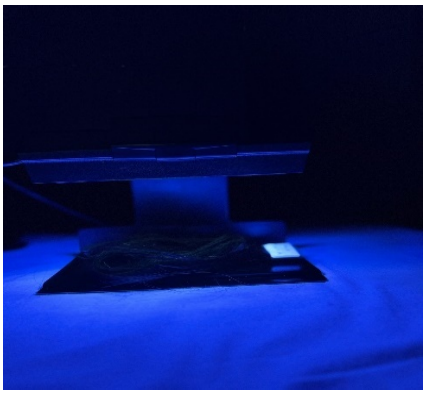

Figure 3: UV damage

TESTRONIX Tensile Strength Tester Automatic Combing System - Fatigue Test

After completion of the 12 treatment/wash cycles, all hair swatches were subjected to wet fatigue testing using the TESTRONIX Tensile Strength Tester Automatic Combing System. The instrument was employed to evaluate hair fiber breakage under standardized laboratory conditions.

A total of 25 combing strokes was applied to each swatch at a controlled rate of 1 stroke per minute with a 10-gram load. Only broken hair fibres were included in the analysis. To avoid the inclusion of naturally shed full-length hairs, a cut-off of three-fourths (¾) of the full hair length was applied.

All procedures were carried out under controlled environmental conditions (temperature: 20–25 °C; relative humidity: 50–60%) at the in vitro testing facility of NovoBliss Research. The study adhered to validated internal protocols, ensuring technical accuracy, repeatability, and reproducibility of the results.

After completion of 12 application cycles, all swatches were subjected to a wet fatigue test consisting of 150 strokes using an automated combing machine. Broken hair fibers were collected, measured, and categorized into:

- Short: <6.25 cm
- Medium: 6.25–12.5 cm
- Long: >12.5 cm

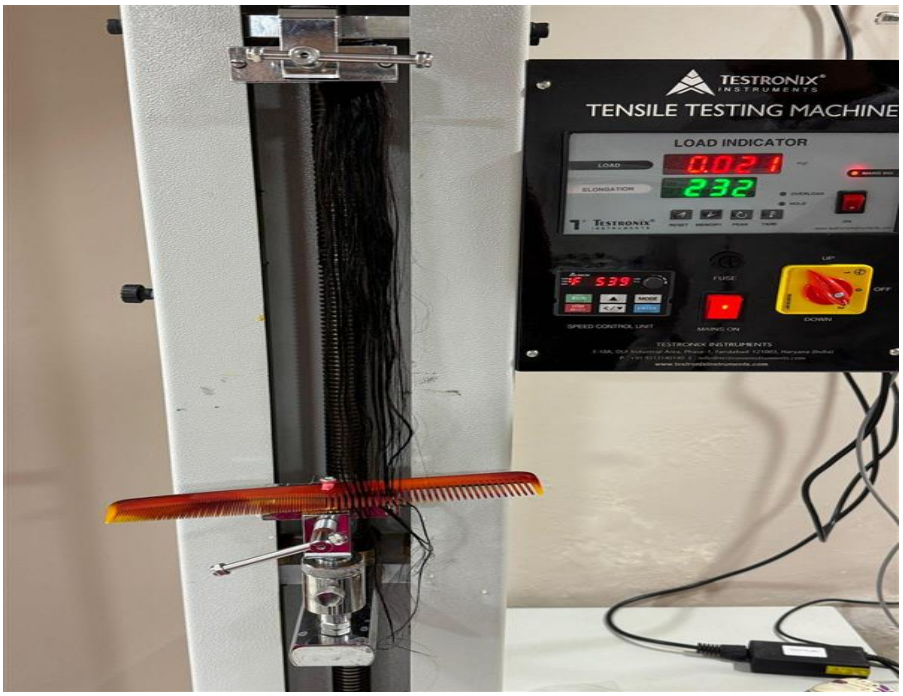

Figure 4: TESTRONIX Tensile Strength Tester Automatic Combing System

Statistical Analysis

Instrumental data were exported to Microsoft Excel, validated, and locked before analysis. Results were expressed as Mean ± SD. Paired two-tailed t-tests were applied to compare the test and control groups, done by using SPSS software (Version 29.0.1.0 (171)) or similar software, with a significance level set at 5% (p-value < 0.05).

This *in-vitro* study employed a 1:1 randomization ratio to allocate hair tresses into two groups: test hair oil and control oil. The randomization sequence was generated using R Software (version 4.3.1, 64-bit) by an independent biostatistician to ensure unbiased group allocation. Double blinding was maintained by providing the study staff who was involved in product application were not involved in any other study-related activities.

Result

Number of Broken Hair

The mean number of broken hair fibers in the Neeli Bhringraj oil group was 11.67 ± 4.46, which was significantly lower compared to the control group (21.67 ± 9.69, p <0.01). This indicates that treatment with Neeli Bhringraj oil effectively minimized hair breakage under fatigue testing conditions.

When expressed in relative terms, the test group demonstrated a 4.19-fold improvement or a 76.11% reduction in hair fall due to breakage compared with the control group. These findings highlight the substantial protective effect of Neeli Bhringraj oil in strengthening hair fibers and preventing damage-induced breakage.

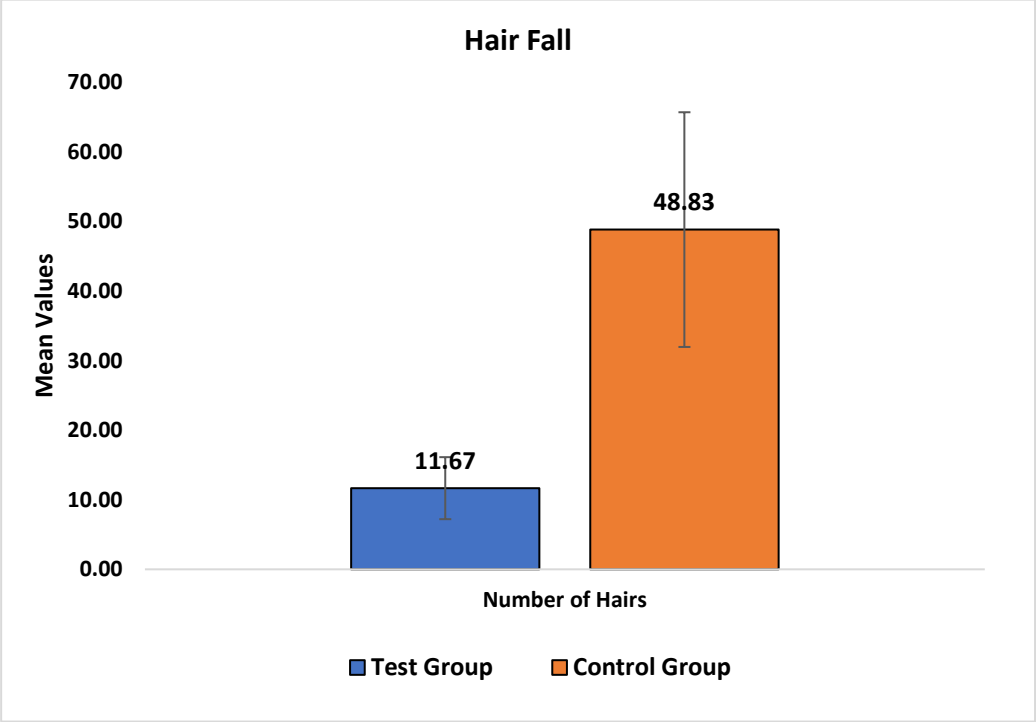

Figure 5: Hair Fall due to Hair Breakage assessed by TESTRONIX Tensile Strength Tester Automatic Combing

Broken Hair Length

Test Group

Broken Hair Length (cm)			
Range	**Below 6.25 cm**	**Between 6.25 to 12.5 cm**	**Above 12.5 cm**
Test Group			
Swatches#01	3(50%)	0(0%)	3(50%)
Swatches#02	8(42.11%)	3(15.79%)	8(42.11%)
Swatches#03	3(23.08%)	2(15.38%)	8(61.54%)
Swatches#04	5(38.46%)	1(7.69%)	7(53.85%)
Swatches#05	2(20%)	3(30%)	5(50%)
Swatches#06	4(44.44%)	3(33.33%)	2(22.22%)
Control Group			
Swatches#07	11(17.46%)	12(19.05%)	40(63.49%)
Swatches#08	15(25%)	7(11.67%)	38(63.33%)
Swatches#09	15(33.33%)	6(13.33%)	24(53.33%)
Swatches#10	13(32.5%)	8(20%)	19(47.5%)
Swatches#11	13(20.31%)	21(32.81%)	30(46.88%)
Swatches#12	6(28.57%)	5(23.81%)	10(47.62%)

The distribution of broken hair fibers across different length categories demonstrated the protective effect of Neeli Bhringraj oil on hair strength. Swatch #01 showed an equal

proportion of short (<6.25 cm; 50.00%) and long (>12.5 cm; 50.00%) fragments, with no medium-length breakage. Swatch #02 had 42.11% short, 15.79% medium, and 42.11% long fragments, while Swatch #03 exhibited 23.08% short, 15.38% medium, and predominantly long fragments (61.54%). Similarly, Swatch #04 showed 38.46% short, 7.69% medium, and 53.85% long fragments. In Swatch #05, the majority of broken fibers were long (50%), with 20% short and 30% medium fragments. Swatch #06 displayed 44.44% short, 33.33% medium, and 22.22% long fragments.

Collectively, these findings indicate that although breakage occurred, the majority of fragments in the test group were of longer length (>12.5 cm), while the proportion of short fragments (<6.25 cm), which signify severe cuticle and cortical damage, was comparatively lower. This shift in breakage pattern toward longer fragments suggests that Neeli Bhringraj oil not only reduced the overall incidence of breakage but also improved the tensile resilience of hair fibers, thereby minimizing severe structural damage.

Control Group

In the control group, the distribution of broken hair fibers indicated higher susceptibility to damage across all swatches. Swatch #07 showed 17.46% short fragments (<6.25 cm), 19.05% medium fragments, and 63.49% long fragments (>12.5 cm). Swatch #08 demonstrated 25% short, 11.67% medium, and 63.33% long fragments, while Swatch #09 exhibited the highest proportion of short fragments (33.33%) along with 13.33% medium and 53.33% long fragments. Swatch #10 also revealed substantial short-length breakage (32.5%), 20% medium, and 47.5% long fragments. Swatch #11 presented 20.31% short, 32.81% medium, and 46.88% long fragments, whereas Swatch #12 showed 28.57% short, 23.81% medium, and 47.62% long fragments. Collectively, the control group displayed a consistently higher proportion of short-length breakage (<6.25 cm) compared to the test group, indicating more severe cuticular and cortical damage.

The presence of greater medium-length breakage in certain swatches further underscores the vulnerability of untreated hair fibers. Overall, these findings suggest that hair treated only with the vehicle control was less resistant to mechanical stress, resulting in greater incidence of severe and moderate breakage compared to the Neeli Bhringraj oil-treated swatches.

Discussion

The present in vitro study demonstrated that repeated application of Neeli Bhringraj oil significantly reduced hair fall due to breakage when compared with the vehicle control. Hair swatches treated with the oil exhibited markedly fewer broken fibers following fatigue testing, underscoring the product's protective and strengthening effects.

The improvement observed with the test treatment can be attributed to multiple mechanisms. Neeli Bhringraj oil is traditionally recognized for its nourishing and strengthening properties, which are supported by experimental evidence. The formulation likely functions by coating the hair shaft, replenishing lost lipids, and providing a protective barrier against environmental stressors such as UV radiation, heat exposure, and pollution. This protective effect reduces cuticle damage, thereby maintaining the integrity and tensile strength of the hair fibers.

Furthermore, the significant reduction in short-length hair breakage in the treated group indicates an enhancement of structural resilience at the fiber level. By preventing fragmentation

and breakage, Neeli Bhringraj oil not only reduces visible hair fall but may also contribute to improved manageability and overall cosmetic appearance of the hair.

The findings of this study are consistent with earlier reports on the role of herbal oils in hair care, particularly formulations enriched with traditional Ayurvedic actives. Unlike simple vehicle-based treatments, the test oil demonstrated clear efficacy, thereby validating the rationale for its use as a functional hair care product.

The study design incorporated controlled laboratory damage models, repeated treatment cycles, and objective fatigue testing using validated instrumentation, ensuring reproducibility and reliability of results. The double-blind protocol minimized bias in the assessment of outcomes, further strengthening the credibility of the findings.

Although the in vitro design provides strong preliminary evidence, it does not account for biological factors such as scalp physiology, sebum secretion, or follicular activity. Therefore, future clinical studies are warranted to translate these findings into real-world consumer benefits.

Conclusion

This in vitro study demonstrated that Neeli Bhringraj oil provided superior protection against hair breakage and significant improvement in hair strength compared with the vehicle control. The observed reduction in short-length breakage and shift toward longer fragments indicate enhanced tensile resilience and minimized structural damage.

These findings scientifically validate the traditional use of herbal oil and establish it as an evidence-based, plant-derived solution for reducing hair fall and improving overall hair health. With its dual action of nourishment and protection, herbal oil represents a safe, natural, and effective intervention with strong potential for both therapeutic and consumer hair care applications.

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Conflict Of Interest

The authors declare that there is no conflict of interest regarding the publication of this research work.

Author Contribution

Dr. Maheshvari Patel conceptualized and supervised the study, contributed to the study design, and coordinated the overall research as part of her PhD work, while also serving as the corresponding author. Dr. Nayan Patel provided strategic oversight and administrative support as Chief Executive Officer of NovoBliss Research Private Limited. Dr. Apeksha Merja

contributed her clinical expertise in dermatology and assisted in reviewing and interpreting the findings in relation to hair health. Dr. Ravikumar R. Patel guided the pharmacological aspects of the work, provided academic supervision, and reviewed the manuscript for scientific accuracy. All authors contributed to manuscript revisions, read, and approved the final version of the article.

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