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abstract

Hair loss and thinning are persistent challenges for consumers seeking effective yet convenient solutions. While leave-on treatments promise strong efficacy, rinse-off formats are often perceived as less potent due to limited contact time. This study evaluated the performance of a bioactive complex based on pumpkin (*Cucurbita pepo*) seed extract, incorporated into a shampoo formulation. Over a 180-day period, treatment with 1 % active ingredient delivered clinically significant improvements compared to placebo, including improved hair density, healthier follicular activity, and better hair cycle balance. Volunteers experienced a visible reduction in shedding and an acceleration of natural growth, resulting in longer, fuller hair and improved scalp coverage. The findings confirm that a rinse-off formulation can deliver clinically relevant outcomes when powered by an active designed for strong molecular interactions and nutrient support. This innovation bridges the gap between consumer convenience and proven efficacy, positioning rinse-off products as a new generation of treatment-oriented hair care solutions.

Introduction

Hair growth and anti-hair loss remain top consumer priorities, driven by stress, aging, and lifestyle factors that accelerate thinning and shedding [1]. Leave-on formulations have traditionally dominated this space because they allow prolonged contact time for actives to penetrate and act on the follicle. Published in *Cosmetics & Toiletries* (March 2023) [2], pumpkin (*Cucurbita pepo*) seed extract (HAIRVIVINE®-PRO) demonstrated strong efficacy in leave-on applications: scalp studies showed up to 14.5% increase in hair density, 32% reduction in hair loss, and visible improvements in hair volume and growth perception. Eyelash studies confirmed similar benefits, with significant gains in length, diameter, and volume. Mechanistically, these effects are linked to activation of autophagy and improved mitochondrial fitness, which are critical processes for keratinization and healthy hair shaft formation.

However, consumers increasingly seek rinse-off solutions (shampoos, conditioners) that combine convenience with performance. Pain points include skepticism about efficacy due to short contact time, desire for multifunctional products (cleansing + treatment), and preference for lightweight, non-greasy formats. Although rinse-off products have limited contact time, studies show they still leave measurable residues on hair and skin. Deposition efficiency depends on ingredient affinity to keratin and cuticle surfaces, influenced by charge, hydrophobicity, and molecular interactions. Conditioning agents and functional actives often rely on hydrogen bonding with hydroxyl groups in keratin and polar sites in the cuticle, creating temporary but stable associations that persist after rinsing. Advanced imaging confirms that shampoo and conditioner residues remain on the scalp and adjacent skin for up to two hours post-wash, enabling continued functional benefits. Beyond surface deposition, hair

follicles act as micro-reservoirs where actives can diffuse into the follicular duct and penetrate deeper layers, enabling prolonged availability even after rinse-off. Also, mechanical stimulation during washing, such as shampooing, can enhance follicular penetration by pushing actives deeper into the duct and improving their distribution. These mechanisms highlight that even short-contact formulations can achieve meaningful deposition and performance when designed for strong molecular interactions [3-6]. Addressing these needs requires proving that pumpkin seed extract can deliver measurable hair growth and anti-hair loss benefits even under rinse-off conditions.

This study explores the impact of pumpkin (*C. pepo*) seed extract in a rinse-off formulations, aiming to elucidate its ability to support hair growth and reduce hair loss despite limited exposure time, bridging the gap between consumer convenience and proven efficacy.

Materials and methods

To assess the effectiveness of pumpkin seed extract, in a placebo-controlled rinse-off application (washing every second day) for anti-hair loss and hair growth, 54 people with hair loss (25 male and 29 female) completed the study. Subjects have been instructed not to wash their hair 24h before visit at D-2, D0, D88, D90, D178, and D180. In order to quantify the hair growth rate, at D-2, D88 and D178 a small area has been shaved in all volunteers and pictures with PhotoMAX Pro have been taken. At D0, D90 and D180 data collection occurred including 60 times combing method, PhotoMAX Pro, Canon EOS 5D Mark IV. Data have been analysed in case of normal distribution

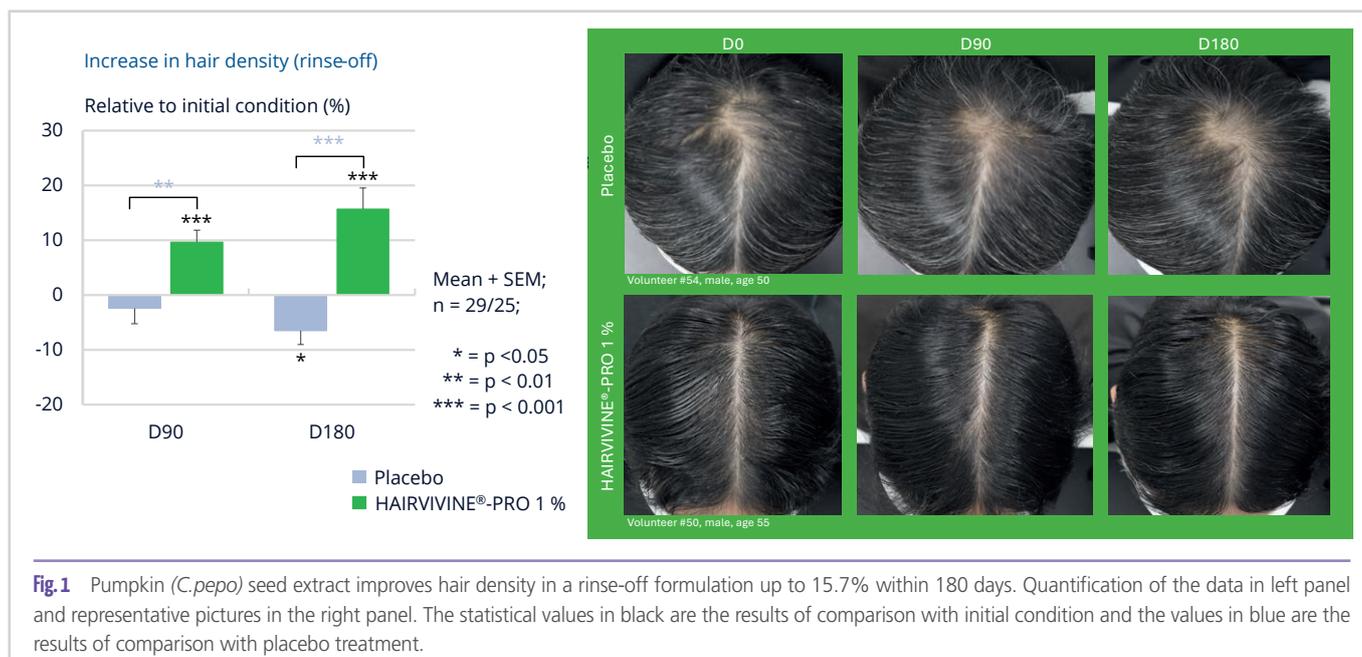


Fig. 1 Pumpkin (*C.pepo*) seed extract improves hair density in a rinse-off formulation up to 15.7% within 180 days. Quantification of the data in left panel and representative pictures in the right panel. The statistical values in black are the results of comparison with initial condition and the values in blue are the results of comparison with placebo treatment.

with a Student’s t-test otherwise, the rank-sum test was used. Two-tailed test was used for all statistical methods.

Results

Hair density analysis demonstrated contrasting results between the placebo and treatment groups. The placebo group showed a progressive decline, reaching a significant 6.5% decrease after 180 days compared to baseline. In contrast, treatment with 1% pumpkin (*C.pepo*) seed extract led to a marked improvement in hair density, with an average increase of 9.7% after 90 days and 15.7% after 180 days, both statistically significant compared to baseline and placebo (**Figure 1**). These findings indicate that pumpkin (*C.pepo*) seed extract not only prevents density loss but actively promotes hair growth over time.

The vellus-to-terminal hair ratio is a critical parameter for assessing follicular health and hair density. Vellus hairs

are short, fine, and non-pigmented, typically measuring less than 30 μm in diameter, whereas terminal hairs are thicker (> 60 μm), pigmented, and longer. A healthy scalp generally exhibits a low vellus ratio, reflecting robust follicular activity. An increased ratio often indicates follicular miniaturization, a hallmark of androgenetic alopecia and other pathological conditions. In our study, treatment with 1% pumpkin (*C.pepo*) seed extract resulted in a significant reduction of vellus hair, approximately 5%, after just 90 days, with this improvement maintained throughout the treatment period. In contrast, the placebo group returned to baseline levels by day 180 (**Figure 2, left**). In parallel anagen/telogen ratio compared to baseline and placebo, reached 3.04% after 90 days and 3.93% after 180 days, demonstrating enhanced follicular activity and improved hair cycle homeostasis (**Figure 2, right**).

Hair loss among volunteers treated with 1% pumpkin (*C.pepo*) seed extract has been analyzed by combing method, which revealed a significant reduction compared to both baseline and placebo. After 90 days of treatment, the verum group

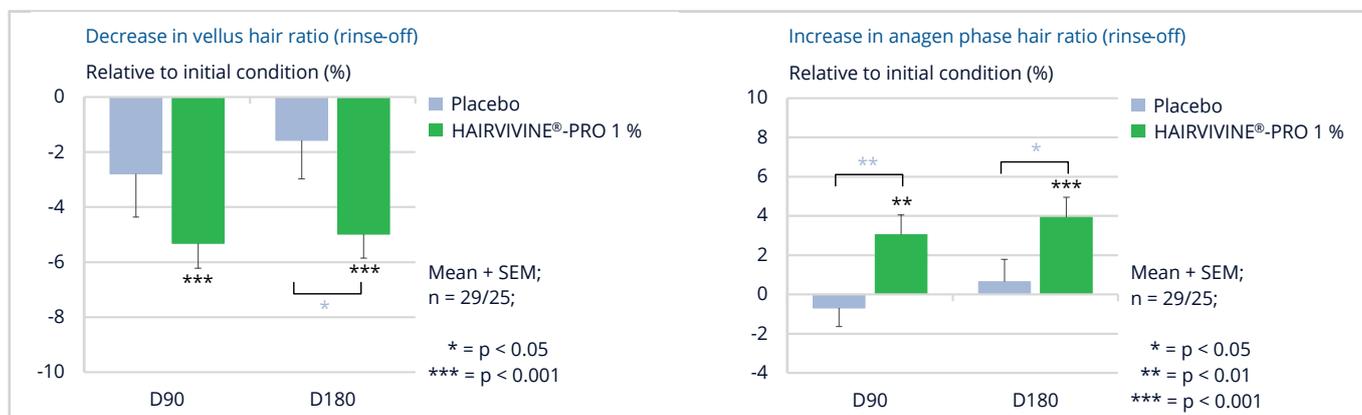


Fig. 2 Pumpkin (*C.pepo*) seed extract reduces vellus hair ratio and increases anagen phase ratio. Application of a shampoo every second day containing 1% pumpkin seed extract significantly reduced vellus hair ratio (5%) and increased anagen phase ratio up to 3.9% after 180 days. The statistical values in black are the results of comparison with initial condition and the values in blue are the results of comparison with placebo treatment.

demonstrated a decrease of 2.6%, which further improved to 4.9% by day 180. In contrast, the placebo group exhibited minimal changes, remaining below 1% throughout the study period. These findings indicate that pumpkin (*C.pepo*) seed extract in a shampoo formulation effectively reduces hair loss over time, whereas placebo treatment shows no clinically relevant impact (Figure 3).

Hair growth rate in the Asian population is approximately 0.3 – 0.4 mm/day, which is higher than that of Caucasian hair (~0.3 mm/day) and African hair (~0.25 mm/day). Over-all, Asian hair grows 10 – 15% faster than Caucasian hair and 30 – 40% faster than African hair, primarily due to differences in follicular structure and anagen phase duration [7]. In this study, treatment with 1% pumpkin (*C.pepo*) seed extract significantly enhanced the growth rate, increasing by 0.02 mm/day after 90 days and 0.06 mm/day after 180 days compared to baseline. In contrast, the placebo group exhibited a negative trend, with a reduction of up to 0.02 mm/day at both measurement points. These findings confirm the efficacy of HAIRVIVINE®-PRO in promoting hair growth beyond the physiological baseline (Figure 4). After 180 days, hair length in the pumpkin (*C.pepo*) seed extract group should be 7.38 cm, compared to 6.30 cm at baseline and 5.94 cm in the placebo group, corresponding to an additional 1.08 cm over baseline and 1.44 cm over placebo.

In addition, the proportion of parting line area (seam area) has been analyzed. The treatment with 1% pumpkin (*C.pepo*) seed extract significantly reduced the proportion of parting line area by 2.8% after 90 days and 4.8% after 180 days compared to baseline, indicating improved coverage and follicular activity. In contrast, the placebo group showed a negative trend, with a reduction of up to 1% at day 180 (data not shown).

Conclusion

The results clearly demonstrate the efficacy of 1% pumpkin (*C.pepo*) seed extract in a rinse-off shampoo formulation for improving multiple hair health parameters. Unlike the placebo

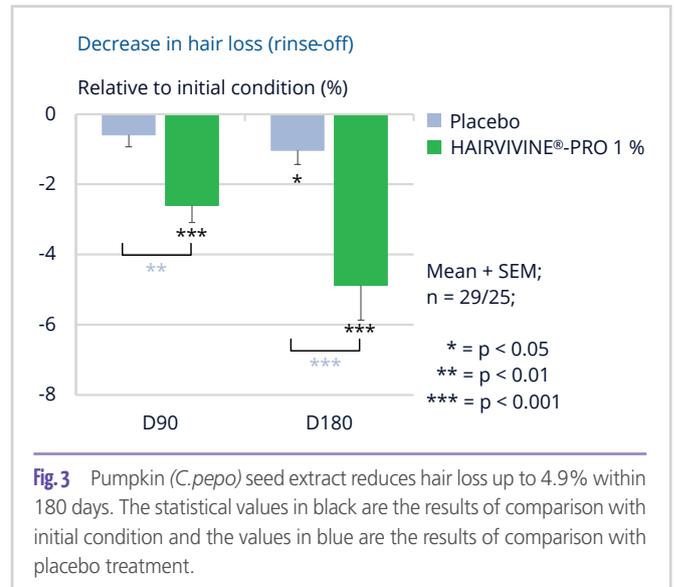


Fig. 3 Pumpkin (*C.pepo*) seed extract reduces hair loss up to 4.9% within 180 days. The statistical values in black are the results of comparison with initial condition and the values in blue are the results of comparison with placebo treatment.

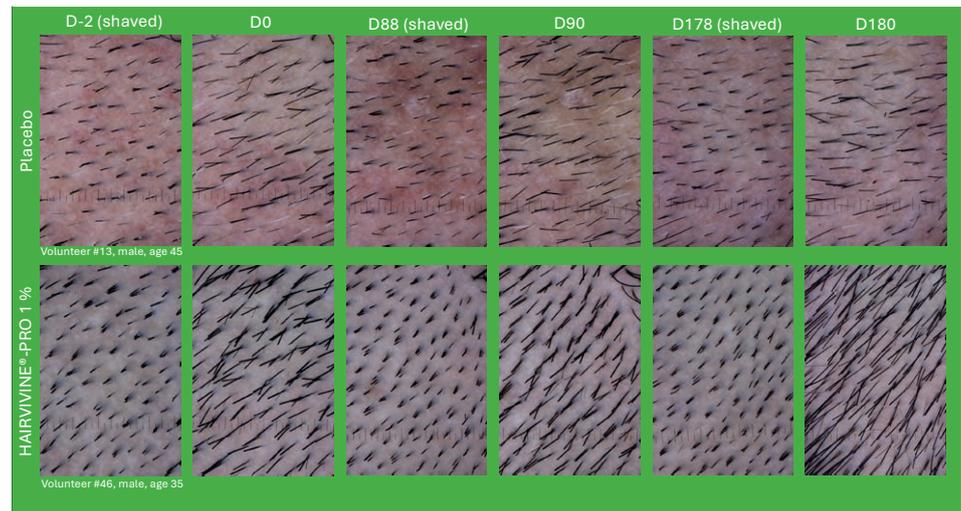
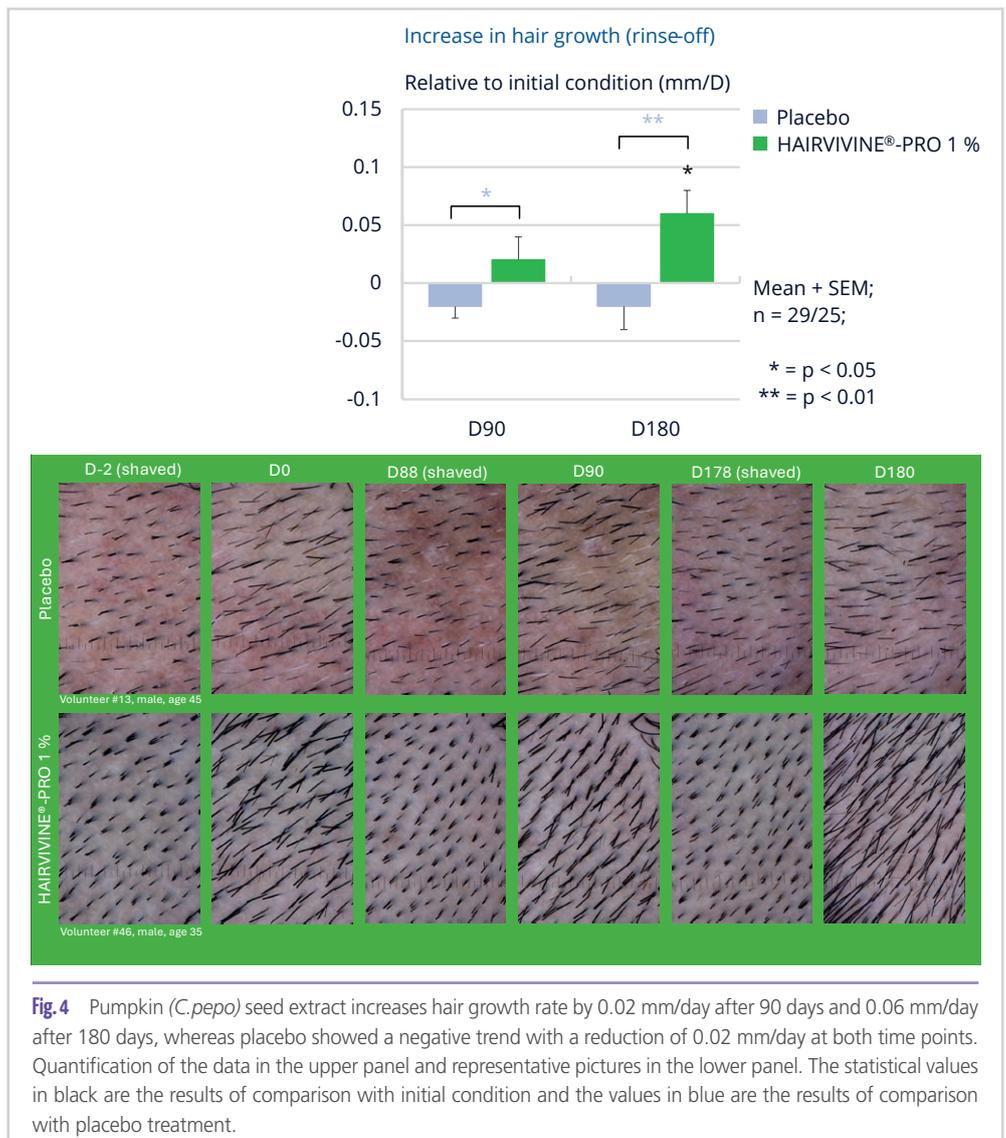


Fig. 4 Pumpkin (*C.pepo*) seed extract increases hair growth rate by 0.02 mm/day after 90 days and 0.06 mm/day after 180 days, whereas placebo showed a negative trend with a reduction of 0.02 mm/day at both time points. Quantification of the data in the upper panel and representative pictures in the lower panel. The statistical values in black are the results of comparison with initial condition and the values in blue are the results of comparison with placebo treatment.

group, which showed a progressive decline in hair density (-6.5% after 180 days), the treatment group achieved a significant +15.7% increase in hair density over the same period,

confirming active stimulation of hair growth rather than maintenance. Follicular health improved markedly, as evidenced by a 5% reduction in vellus hair ratio and a higher anagen/telogen ratio, indicating enhanced hair cycle homeostasis. Hair loss decreased significantly (-4.9% after 180 days), while growth rate surpassed physiological norms, adding 1.08 cm over baseline and 1.44 cm over placebo after six months. Furthermore, improved coverage was reflected in a 4.8% reduction in parting line area, reinforcing the visual benefits of treatment.

Collectively, these findings confirm that pumpkin *Cucurbita pepo* seed extract in a rinse-off format not only prevents hair density loss but actively promotes growth, strengthens follicular activity, and reduces shedding. These results confirm that short-contact formulations can achieve clinically relevant outcomes when powered by actives designed for strong molecular interactions and follicular support. The findings redefine the performance potential of rinse-off products, bridging the gap between consumer convenience and high efficacy. For manufacturers, this opens opportunities to position shampoos and conditioners as true treatment formats, not just cleansing agents. The ability to deliver measurable hair growth benefits in a daily-use product aligns with market trends favouring multifunctionality, time-saving routines, and natural actives. Brands can leverage this innovation to differentiate in a competitive segment, meet rising demand for anti-hair loss solutions, and strengthen claims with robust scientific backing. RAHN-Cosmetic Actives transform rinse-off hair care from a commodity into a premium, results-driven category.

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