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"Comprehensive Clinical & Morphological Evaluation of A Potent Eyelash Serum: Synergistic Effect of Advanced Peptide and Botanical Extracts to Lash Growth, Density and Overall Appearance"

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1. Introduction

Beyond their physiological purpose of protection, eyelashes are essential to enhance facial attractiveness and confidence of women [1]. Prominent and enhanced eyelashes are highly valued by most cultures, especially in the Arab and Southeast Asian population [2]. Long and dense eyelashes also give desired perception of femininity and youthfulness [3]. Inherent or acquired eyelash loss can cause unnatural appearance, discomfort and low self-esteem while eyelash growth has a positive psychological perception [4].

There are various available methods for eyelash enhancement, such as mascara, artificial eyelashes, eyelash extensions, eyelash lifting, and eyelash dyeing / tinting [5]. However, these methods don't provide continuous or long-term effects, may induce potential side effects, and some techniques depend on the skill of the practitioner / surgeon with no standard procedures. Mascara has a possible ingredient-specific safety concern especially for sensitive eyes and some water-based formulations carry a risk of bacterial and fungal contamination [6]. Artificial eyelashes and eyelash extensions utilize acrylate adhesive found to contain allergy provoking substances such as formaldehyde that reported can cause light adverse effect such as dry eyes, redness to some serious ocular disorders including contact dermatitis, conjunctival erosion and allergic blepharitis [7, 8, 9]. Eyelash lifting lacking preliminary check of blepharitis / meibomitis beforehand has been reported to cause infection due to solutions used during procedure and trigger conjunctivitis and preseptal cellulitis [10]. Dyes used in lash-tinting treatment which contain common sensitizer in hair dyes such as P-phenylenediamine (PPD) has reported to cause eyelashes loss, inflammatory responses to the lid, conjunctiva and even can cause ocular erosions and corneal ulcerations [11, 12]. Usage of black henna as natural sources of eyelash tinting also has been reported to result in periocular / periorbital contact dermatitis and blepharoconjunctivitis [13]. A safer and more natural alternative approach for augmenting and enhancing eyelashes are eyelash serums.

Eyelash serums are products containing biologically active molecules with various aims to enhance the appearance of eyelash attributes (length, thickness, strength, luster) and may also be used to regrow lashes after disease or insult [14]. The market for eyelash serum has expanded significantly in recent years. At a cumulative annual growth rate (CAGR) of 9.8%, it will increase from $1.14 billion in 2024 to $1.25 billion in 2025 globally. Asia-Pacific was the

largest market for eyelash serum in 2024 [15]. Based on biologically active chemicals, eyelash serums are classified into two categories: prostaglandin analogs and non-prostaglandin analogs.

Prostaglandins are biological mediators made of the eicosanoid family which act as local hormones. Among prostaglandins, the PGE2 and PGF2α were described as possible modulators of hair growth. Local prostaglandin production by keratinocytes in skin are suggested to somehow be involved in hair growth control and differentiation [16, 17]. Some synthetic prostaglandin analogs are used to treat ophthalmic conditions (glaucoma, ocular hypertension) and later were found to induce eyelash growth which led to findings of benefit in curing hypotrichosis conditions [18, 19]. Examples of commonly-prescripted synthetic prostaglandin analogs products used for eyelash growth are bimatoprost, latanoprost, fluprostenol, travoprost, tafluprost, dechloro ethylcloprostenolamide, and isopropyl cloprostenate [14]. Bimatoprost is the only (Food and Drug Administration (FDA) approved molecule for eyelash growth. The mechanism of prostaglandin analogs to induce eyelash growth works through extension of anagen phase to increase length and induction of anagen phase restart in telogen-phase follicles [20, 21]. Eyelash hypertrichosis is a commonly reported adverse effect of prostaglandin analogs since their introduction in the 1990s [22]. Other reported side effects of prostaglandin analogs are eyelid pigmentation, hyperemia (excessive dilation of blood vessels), irritation, dry eyes, discharge [23]. Proper application is needed to isolate the use only on eyelashes because evidence reports that direct contact to the eye can cause permanent iris pigmentation by mechanism of melanogenesis of melanocytes, especially in light colored eyes users [24].

Non-prostaglandin analogs for eyelash enhancement include growth factors, phytochemicals, peptides, vitamins, amino acids and synthetic therapeutic drugs [14]. These active molecules are proven by some studies that present promising mechanisms for enhancing eyelashes. Growth factors are specific proteins that regulate cell growth, proliferation, differentiation and survival by signaling molecules that bind to specific receptors. Due to its high molecular weight, penetration to epidermis is challenging hence the delivery by injectable formulation is emerging. The mechanism of growth factor works through targeting various levels of growth cycle, stimulating proliferation and modulating growth-phase transition at the bulge stem cells level in dermal papilla [25]. An eyelash serum of polygrowth factor containing keratinocyte, fibroblast, insulin-like, vascular endothelial growth factor was reported to have efficacy of improving eyelash length, luster, thickness, volume, color, and curl [26]. Some phytochemicals used as active molecules in eyelash serum are ginseng root extract, pumpkin seed oil, Coffea arabica and Larrea divaricata (Jarilla) extract and castor oil. Ginseng contains bioactive constituents especially saponin compounds known as ginsenosides which have been proposed to have hair-growth potential. Most frequently used ginseng among various species are Panax ginseng (Korean or Asian red ginseng). The mechanism of ginseng root extract in eyelash growth is reported to be similar to prostaglandin analog by induction and prolongation of anagen phase while decreasing catagen phase [27]. Another mechanism proposed are inhibitory effects against activity of 5α-reductase, a testosterone-mediated hair growth suppressant which is induced by ginsenoside Ro, Rg3 and Rd. Red ginseng oil (RGO) were reported to significantly regenerate major components of hair regenerative capacity such as linoleic acid and β-sitosterol while red ginseng extract (RGE) protect hair matrix keratinocyte proliferation against dihydrotestosterone (DHT)-induced suppression [28, 29, 30]. Pumpkin seed oil were proposed to promote hair growth by mechanism of inhibitory effects against activity of 5α-reductase with its antioxidant and anti-inflammatory properties. Studies also shown its efficacy on decreasing hair shaft diversity in thickness significantly and increasing mean hair count [31, 32]. Jarilla-Coffea extract, popular in Argentina for treating alopecia, showed an efficacious effect on increasing thickness in eyelashes [33]. Castor oil, well-known vegan active in eyelash serum, were proposed to enhance eyelashes through a mechanism of increasing prostaglandin production by increase of ricinoleic acid [34]. Peptides are already

a popular active use in hair growth products, but its efficacy study on eyelashes is limited. Some examples of peptides used in eyelash serums are myristoyl hexapeptide-16, myristoyl pentapeptide-17, and acetyl tetrapeptide-3. Peptides works through stimulation of keratin production, induction of hair follicle dermal papilla cell growth and production of various growth factors such as vascular endothelial and fibroblast growth factor [35]. A randomized controlled trial study on combination of biochanin A, acetyl-tetrapeptide-3 and ginseng extracts on androgenic alopecia patients show comparable efficacy to minoxidil, a synthetic drug commonly also used in hair loss treatment [36]. Panthenol, a derivative of vitamin B5 majorly thought as treatment of hair breakage and loss, are also believed to have efficacy on eyelash growth. Its mechanism involves increasing cellular proliferation markers in dermal papilla cells, hence increasing cell viability and prolonging anagen phase [37]. Biotin (vitamin B7 or vitamin H), a rising ingredient in eyelash serum, is an important molecule needed in a very small quantity for metabolism of amino acids, carbohydrates and fatty acids that impact hair, skin and nail health. A study reported that a patient suffering from biotinidase deficiency showed alopecia including lack of eyebrow and eyelashes. After 5 months of biotin treatment, reappearance of hair including eyebrow and eyelashes took place [38]. Proline, a cyclic amino acid commercially used as active in OTC eyelash serum, is thought to have an analogous mechanism on eyelashes by arranging the rate of collagen synthesis that will strengthen hair structure and quality [39].

Compared to commonly-prescripted prostaglandin analogs that may have widely reported of its adverse effects, non-prostaglandin analogs present a vast option and promising results as a safer alternative. However, studies of non-prostaglandin analogs assessment are currently mainly limited to focus on hair / scalp efficacy. There is a lack of formal studies evaluating the safety and quantitative effectiveness of these ingredients for eyelash enhancement, shown by report of Baiyasi et al. through assessment of Oxford Centre for Evidence-Based Medicine that give Level 5 (lowest level of evidence, by expert opinion without explicit critical appraisal) and Grade D (very low-quality evidence with a weak recommendation) on many non-prostaglandin analogs actives [14].

2. Materials and Methods

A novel water-based serum was formulated by combining 8 botanical extracts (Scutellaria Baicalensis root, Glycine soja germ, Triticum vulgare germ, Trifolium pratense flower, Larix europaea wood, tea tree leaf, red ginseng root, Aloe vera leaf) and 2 peptides (acetyl tetrapeptide-3 and biotinoyl tripeptide-1). Humectant and thickener were also incorporated in the formulation to improve sensory feel and enhance penetration. The placebo was prepared with the same formula by taking out the 10 active ingredients.

Comprehensive safety assessments were performed to evaluate the test product's dermal and ocular tolerability. These assessments included the dermatological and ophthalmological evaluations. The dermatological assessment was performed in 21 healthy subjects to ensure its dermal tolerability by observing any unwanted cutaneous reaction that might occur. On the other hand, an ophthalmological assessment was carried out over 28 days in 21 subjects to assess the ocular tolerability. Daily application of the product around the eyes was evaluated for tolerability using clinical parameters, including ocular reactions, tear film break-up time (TBUT), and overall ocular compatibility.

Product performance in enhancing eyelash length and density was assessed by a double-blind, placebo-controlled clinical study. The study was performed in 42 female subjects, aged 22 to 35, screened based on the following visual grading criteria by self-assessment in Figure 1. Subjects with low character of eyelash attributes from length, thickness and curl were chosen to investigate whether the effect of treatment will give more prominent result.

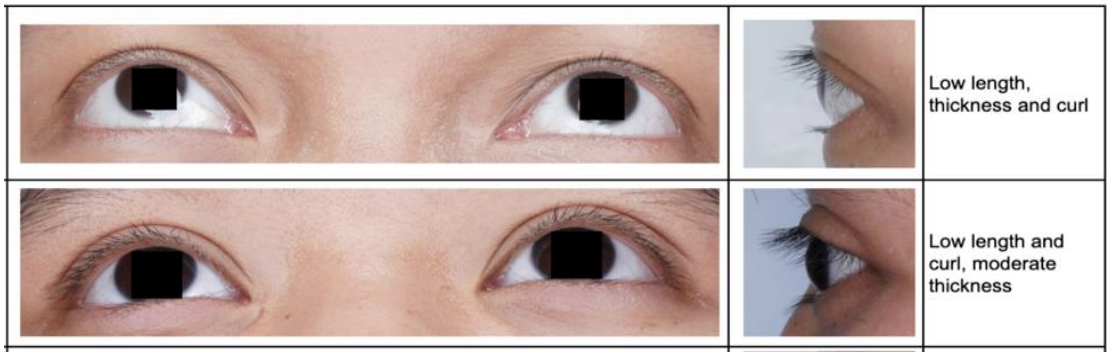

Figure 1. Visual grading criteria of subjects with low character on eyelash attributes

Study subjects were instructed to apply the product twice daily (morning and evening) over a 84-day period. The product performance was evaluated using image analysis (Keyence VHX 700). Data were then analyzed using IBM SPSS Statistics version 29. Before testing the mean difference between two groups, a normal distribution test was carried out. If the data met the assumption of normality, a two-tailed paired samples t-test was used to compare pre-and post-treatment data, or an independent samples t-test was applied for between-group comparisons. If normality requirement was not met, the Wilcoxon signed-rank test or the Mann-Whitney U test was used. A p-value of less than 0.05 was considered statistically significant.

All of the studies were performed according to the Declaration of Helsinki and GCP Guideline (ICH E6 [R1, R2]).

3. Results

Clinical study

After usage of eyelash serum formulation, a significant enhancement of eyelash attributes of length and density observed rapidly since week 2 (Figure 2). Assessment after usage showed significant ($p < 0.001$) increase in length compared to baseline (week 0 before treatment) by 42.28% at week 2, 48.86% at week 4, 57.60% at week 8 and 61.31% at week 12. Placebo without active molecules showed an increase of length by 33.42% at week 2 but the value was not remarkably increased and persisted at 34.58% at week 4, 35.55% at week 8 and 34.32% in week 12. Usage of serum also showed significant ($p < 0.001$) increase in density compared to baseline by 14.84% at week 2, 19.58% at week 4, 22.36% at week 8 and 25.10% at week 12. Placebo showed insignificant ($p > 0.05$) increase of density by 1.05% at week 2, 0.73% at week 4, 0.92% at week 8 and 1.24% at week 12. Visual observation also gave evidence of progressive and visible improvement of overall eyelash appearance (Figure 3). From 2 weeks of usage, the number of eyelash strands increases from areas without growing strands beforehand and eyelashes look thicker, fuller, longer notably after continuous usage of 12 weeks. Statistical analysis comparing serum formulation to placebo also showed that serum formulation significantly presented better result in enhancing eyelash length than placebo starting from week 4 ($p<0.05$) and eyelash density starting from week 2 ($p<0.01$)

Self-assessment questionnaire

From the questionnaire, perception of subjects was assessed by various aspects : preference, sensory feel, safety and result perception (Figure 4). Subjects demonstrated high satisfaction on liking (90%) and interest to buy (86%). On the sensorial aspect, approval rate of > 85% were obtained as the subjects found the formulation easy to apply (fast absorbing, lightweight) and have a likable texture (no tacky, no greasy, feel fresh, hydrating, nourishing and regenerating). On the safety aspect, 95% feels no stinging and 100% no irritation. After 12

weeks, result perception showed approval rate >80% as the subjects perceived healthier looking, longer, denser, more volumized, darker, shinier and stronger eyelashes.

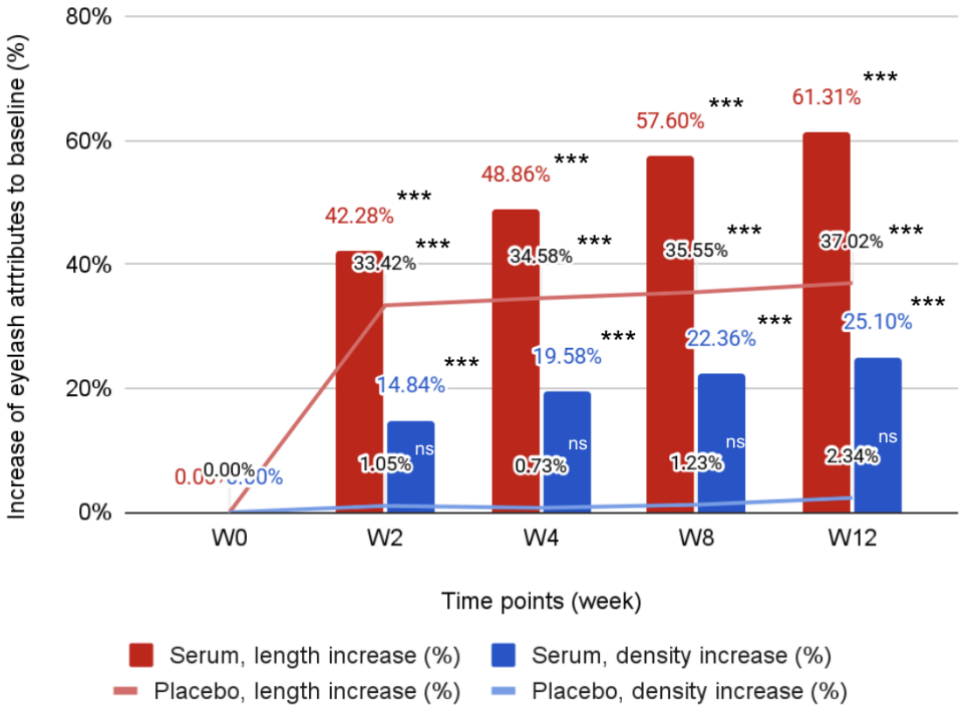

Figure 2. Increase of eyelash attributes after 12 weeks application of eyelash serum (***, $p < 0.001$) and placebo (ns, $p > 0.05$), compared to baseline before treatment

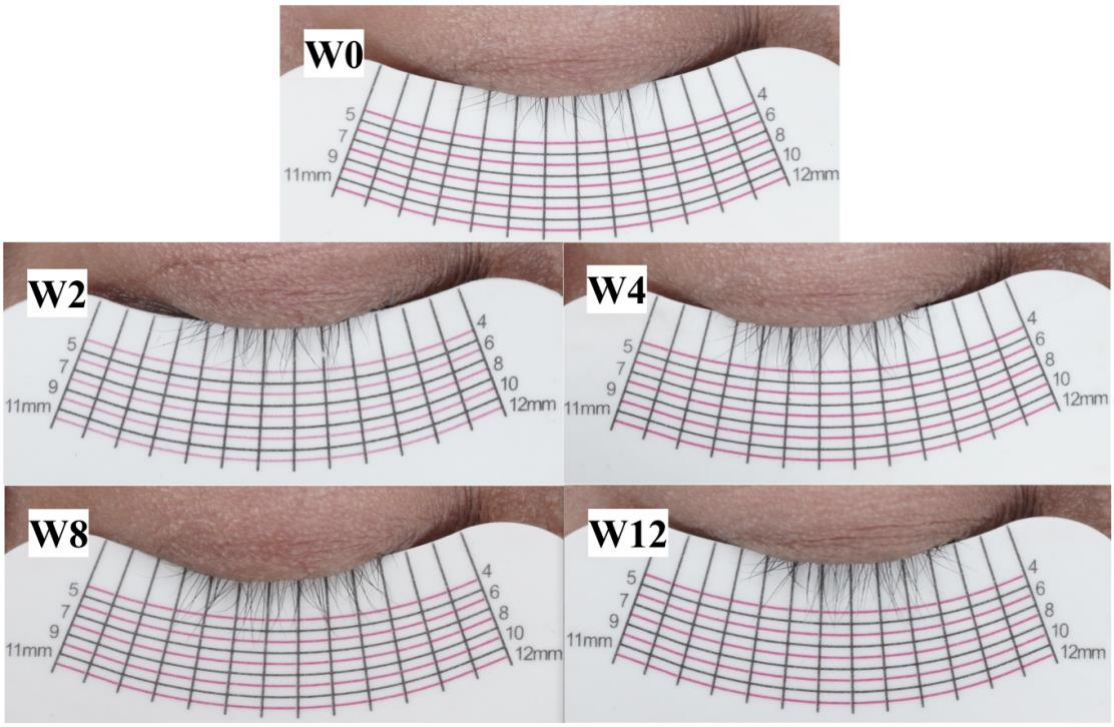

Figure 3. Visual observation of eyelash enhancement after 12 weeks application of eyelash serum

Safety evaluation by dermatologist and ophthalmologist

Dermatologist assessment of clinical signs on safety at 4 weeks observed 1 subject having insignificant squama (+4.76%) and no effects of irritation, allergy, erythema, and edema. The formulation also achieved non-comedogenic and non-acnegenic results by counting non-inflammatory and inflammatory acne lesions, respectively. After 4 weeks, the formulation passed the ophthalmologist assessment by evaluation of tears film break-up time (TBUT) and ocular acceptability. At baseline the formulation induced a slight sign of dryness since there is 1 subject (5%) experiencing abnormal TBUT. However, this sign is not clinically relevant because no adverse event was reported from the subjects. The mean score of global ocular compatibility was 3.9±0.3 (out of 4).

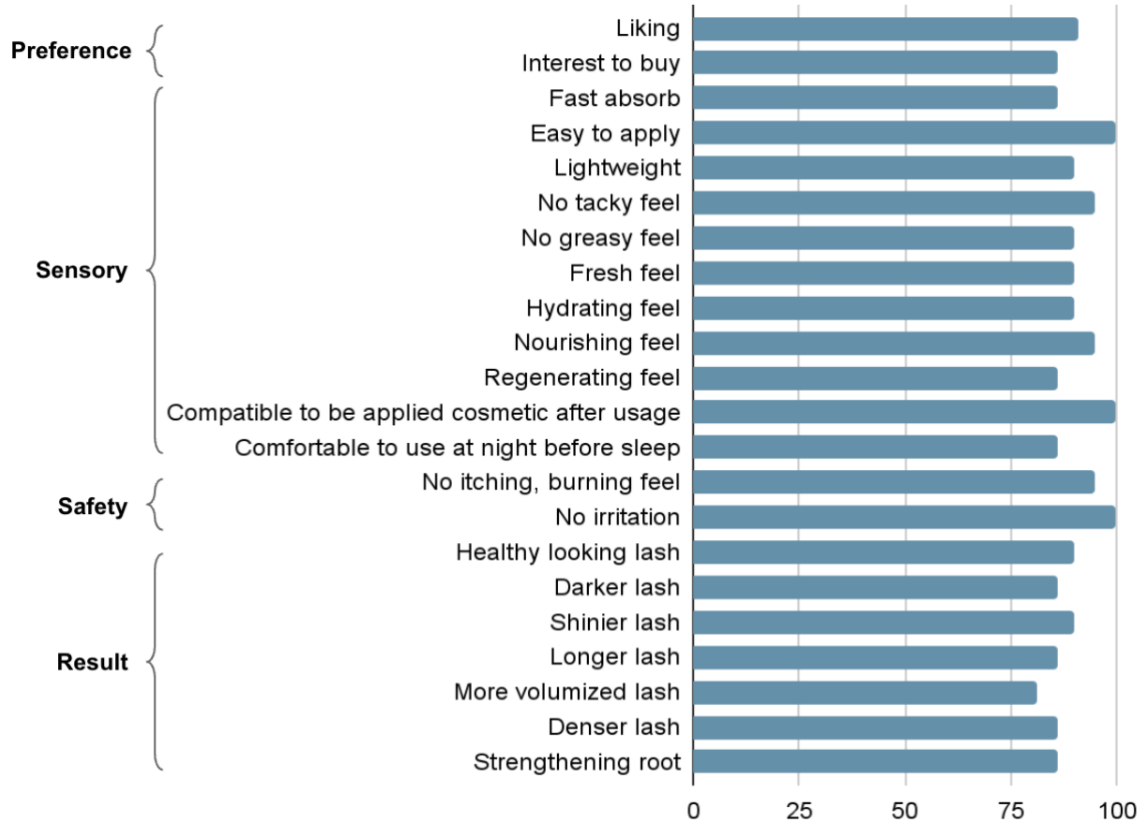

Figure 4. Percentage of approval on self-assessment questionnaire on aspects of preference, sensory feel, safety and result perception.

4. Discussion

The clinical study showed that the combination of botanical extracts and peptides could exhibit rapid enhancement of eyelash growth from the second week of use. By visual observation, the eyelashes become longer and fuller as new roots are filling the upper eyelid margin. Increasing density also gives a blacker overall appearance. Compared to placebo, treatment with eyelash serum showed progressive improvement on length from +42.28% in 2 weeks to +61.31% after 12 weeks of usage while the effect of placebo only showed improvement of length increase of +33.42% in 2 weeks of usage but the the length is constant on +34.32% after 12 weeks. On density, treatment also showed incremental improvement the longer the usage, from +14.84% in 2 weeks to 25.10% in 12 weeks while placebo showed insignificant change constantly from +1.05% in 2 weeks to +1.24% in 12 weeks. From these data, it can

be implied that prolonged usage is recommended to achieve maximum result of length and density.

The serum was formulated by combining active molecules with mechanisms to boost eyelash growth. Results from this study showed an analogous result of hair-targeted ingredients that also have growth activity on eyelash. Scutellaria Baicalensis root, popular as cure of high blood pressure and detoxification contain baicalin, a flavonoid which has the activity to stimulate vascular endothelial growth factor (VEGF) and promote anagen induction in human dermal papilla cells [40, 41, 42]. Both germ extracts from soybean (Glycine soja) and wheat (Triticum vulgare) are rich in glucose that will provide nutrients for cellular energy production, hence inducing longer shafts, increasing hair density and extending anagen phase [43, 44]. Trifolium pratense flowers contain biochanin A, an isoflavone which is an effective inhibitor of 5α-reductase (type I & II) that will hinder hair-growth suppressant enzyme, hence reducing loss of strands. Larix europaea wood and tea tree leaf extract, containing polyphenol such as EGCG (epigallocatechin gallate) and DHQG (dihydroquercetin-glucoside) can improve density by mechanism of inducing proliferation of hair follicle dermal papilla [45]. Red ginseng root extract works through extension of anagen phase and inhibition of hair-growth suppressant [27, 28]. Aloe vera, a popular hair-fall agent can also potentially reduce eyelash breakage [56]. In vivo and ex vivo studies of acetyl tetrapeptide-3 showed its activity of stimulating synthesis of collagen III, collagen VII and laminins that will optimize hair anchorage [46]. Biotinoyl tripeptide-1, a derivative of biotin which is a cofactor of many key metabolic processes impacting hair health, are presumed to have efficacy similar to biotin in providing reappearance of hair including eyebrow and eyelashes [38].

The enhancement of eyelash is supported by the synergistic effect of active molecules in each stage of the eyelash growth cycle. At each anagen phase, baicalin helps to activate stem cells and initiate growth while polyphenol from Larix europaea wood and tea tree leaf induce proliferation of follicle dermal papilla. Hence, new lash strands appear and more density is observed. Longer lash is achieved by support from nutrient provision and extension of anagen phase from glucose, red ginseng root extract and biotin. While at this stage, the growth is maximized by inhibiting hair-growth suppressant from the activity of biochanin A. Hence, anagen stage that is proposed to be between 1-3 months can be fully functioning for increasing length, which can be seen from even more significant results after the usage of serum for 3 months [47]. While at the catagen (transition) and telogen (rest) phase, collagen synthesis from peptides and activity of aloe vera extract help prevent lash shedding and optimize lash root strength.

5. Conclusion

Eyelash serum combining botanical extracts and peptides showed rapid and prolonged enhancement of eyelash by quantitative measurement that is furthermore validated by high satisfaction rates. This study can contribute by filling the gap of formal studies on non-prostaglandin alternatives of safe and efficacious lash enhancement. Further study on the effect of single active materials is needed to validate the efficacy and mechanism in improving eyelash quality.

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