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Study on the plant-derived exosome like nanoparticles essence composition to eliminate crow's feet wrinkles

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1. Introduction

Crow's feet are the wrinkles appear at the side of the temples, stretching out towards the temples. Because the skin around the eye is 40% thiner than skin of other face area, accompanied with the decrease of amount of collagen and elastin, crow's feet begin to form around the eyes. Laughing, concentrating, squinting or frowning and environmental and lifestyle factors can accelerate the crow's feet formation. The methods to treat crow's feet include botulinum toxin injection, skin peel, dermal filler to stimulate collagen production, and cosmetics specialized for crow's feet.

The application of anti-aging essence involves gentle massage around the eye area, ensuring minimal pressure to avoid irritation. Regular use, typically twice daily, is essential for achieving visible results, as the bioactive components require consistent exposure to exert their effects. Key ingredients such as peptides, vitamin C, hyaluronic acid, and retinoids play pivotal roles in reducing the appearance of crow's feet. Peptides signal the skin to produce collagen, strengthening the dermal matrix, while vitamin C promotes collagen synthesis and brightens the skin. Hyaluronic acid hydrates the skin, plumping it and reducing fine lines, whereas retinoids increase cell turnover and collagen production. Additionally, antioxidants like vitamin E and green tea extract neutralize free radicals, mitigating oxidative stress and delaying further aging.

By integrating these mechanisms, anti-aging essence offer a non-invasive and effective strategy to improve skin texture and reduce the visibility of crow's feet, aligning with consumer demands for natural yet potent skincare solutions. Further research into optimized formulations and application protocols may enhance the efficacy of these products, contributing to advancements in anti-aging cosmeceuticals.

Plant-derived exosome-like nanoparticles (PELN) are natural nanocarriers isolated from various plants, including fruits, vegetables, and herbs [1]. These nanoparticles are composed of lipids, proteins, nucleic acids, and bioactive compounds, and they exhibit excellent biocompatibility, low immunogenicity, and enhanced safety. PELNs can be used as delivery systems for active ingredients in cosmetics, improving their stability, penetration, and bioavailability [2-5].

In cosmetics, PELNs have shown promising applications. For example, they can enhance the skin penetration of peptide-based ingredients, which are often limited by their instability and the skin's barrier function. PELNs also possess antioxidant and anti-aging properties, which help reduce oxidative stress and DNA damage caused by UV exposure, thereby delaying skin photoaging. Additionally, PELNs can regulate melanin production, making them potential candidates for skin brightening and anti-pigmentation products. Their ability to carry and deliver microRNAs and other bioactive molecules further expands their potential applications in skincare, such as improving skin repair and regeneration. Overall, PELNs offer a natural and effective approach to enhancing the efficacy of cosmetic products while ensuring safety and sustainability.

2. Materials and Methods

2.1 PELNs extraction

Plant exosomes or plant-derived exosome like nanoparticles were extracted from 5 plants: *Centella asiatica* root, *Panax ginseng* root, *Vaccinium myrtillus* fruit, *Camellia reticulata* flower, and *Avena sativa* germ by high-speed gradient ultracentrifugation method [6]. First, fresh plant materials were washed thoroughly and homogenized in sterile phosphate-buffered saline (PBS, pH 7.4). The homogenate was centrifuged at 1000g for 10 minutes to remove large debris, followed by centrifugation at 10,000g for 30 minutes to eliminate cellular organelles and larger vesicles. The supernatant was then subjected to ultracentrifugation at 100,000g for 2 hours using a Beckman Coulter Optima LE-80K ultracentrifuge equipped with a Type 70 Ti rotor. The resulting pellet, containing PELNs, was resuspended in PBS and stored at 4°C for further characterization.

2.2 PELNs characterization

The PELN particle size and zeta potential was tested by dynamic light scattering (DLS). The hydrodynamic diameter and zeta potential of PELNs were measured using a Zetasizer Nano ZS90 (Malvern Instruments, UK). Samples were diluted in PBS prior to analysis to ensure appropriate particle concentration for measurement.

The morphology and size of PELNs were visualized using TEM. A drop of PELN suspension was placed on a copper grid, negatively stained with 2% uranyl acetate, and imaged using a JEOL JEM-1400 transmission electron microscope at 80 kV.

2.3 PELNs eys essence preparation

The PELNs eys essence was formulated with Centella asiatica root PELN 1.0, Panax ginseng root PELN 1.0, Vaccinium myrtillus fruit PELN 0.5, Camellia reticulata flower PELN0.5, Avena sativa germ PELN 0.5, hyaluronic acid (low molecular weight) 2.0, vitamin C (L-ascorbic acid) 5.0, vitamin E (Tocopherol) 0.5, niacinamide 3.0, caffeine 0.5, glycerin 5.0, cetyl alcohol 1.0, stearyl alcohol 1.0, phenoxyethanol 0.5, ethylhexylglycerin 0.2, purified water q.s. to 100%.

Glycerin, cetyl alcohol, and stearyl alcohol were melted together at 75°C to form the emollient base. Hyaluronic acid, vitamin C, vitamin E, niacinamide, and caffeine were dissolved in purified water at 75°C. The isolated PELNs were added to the aqueous phase while maintaining a temperature below 40°C to preserve their bioactivity. The emollient base and aqueous phase were combined under gentle stirring at 75°C until homogenized. Phenoxyethanol and ethylhexylglycerin were added to ensure product stability and shelf life.
The mixture was cooled to room temperature, and the pH was adjusted to 5.5–6.0 using citric acid or sodium hydroxide if necessary.

2.4 PELNs eye essence skin test

2.4.1 Eye skin test

The eye essence with PELNs was used twice daily, 12 hours apart by 26 volunteers for 42 days. The age of volunteers were 45.2 to 58.5 years, and the gender is female 18, male 8. The essence was applied evenly to the wrinkles on the outside of eyes about 50 μl per side after clean the face.

2.4.2 Measurement of wrinkle depth

Wrinkle depth was quantified using a skin surface texture analyzer (VisioRepertor®; Delfin Technologies, Finland). High-resolution images of the periorbital area were captured under standardized lighting conditions. The images were analyzed using specialized software to determine the average wrinkle depth (μm) based on the vertical distance between the peaks and valleys of the skin surface. Each measurement was performed in triplicate, and the average value was recorded.

2.4.3 Wrinkle Scoring

Wrinkle severity was assessed using a standardized visual scoring system by trained dermatologists. A five-grade scale (0–4) was used: 0=no visible wrinkles, 1=very fine lines, 2=fine lines, 3=moderate wrinkles, and 4=deep wrinkles. Assessments were performed under controlled lighting conditions, and the results were recorded by two independent evaluators to ensure reliability.

2.4.4 Skin moisture content

Skin moisture content was measured using a corneometer (Corneometer® CM 825; Courage + Khazaka Electronic, Germany). The probe was placed gently on the periorbital skin, and readings were taken in triplicate. The moisture content was expressed in arbitrary units (AU), with higher values indicating increased hydration.

2.4.5 Skin elasticity

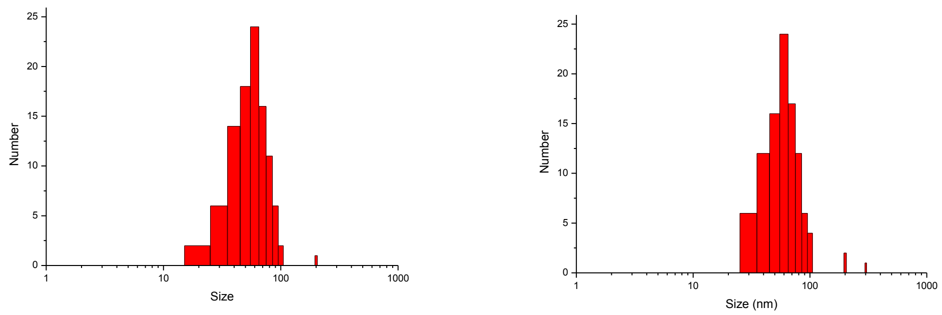
Skin elasticity was evaluated using a cutometer (Cutometer® MPA 580; Courage + Khazaka Electronic, Germany). The probe was applied to the periorbital area, and suction was applied for 2 seconds followed by release. The deformation and recovery of the skin were measured, and parameters such as R2 (net elasticity) and R5 (biological elasticity) were calculated. Measurements were performed in triplicate, and the average values were recorded.

2.4.6 Skin whitening degree

Skin whitening degree was assessed using a skin colorimeter (CR-400; Konica Minolta, Japan). The L* value (lightness) was measured in the periorbital area, with higher L* values indicating lighter skin tone. Measurements were taken in triplicate, and the average L* value was recorded. The whitening effect was calculated as the percentage change in L* value before and after treatment.

3. Results

By ultracentrifugation, plant-derived exosome like nanoparticles of five plants was separated. The particle size of the five PELNs was tested by dynamic light scattering. The PELN particle size vary from 20.0~486.2 nm depending on the plant species (Fig.1), the particle size of the



vast majority of PELNs is between 40 and 80 nm. Average zeta potential varies between -35.8~-4.3 mV. The concentration of PELN were $5.78 \times 10^9 \sim 1.33 \times 10^{10}$ per ml.

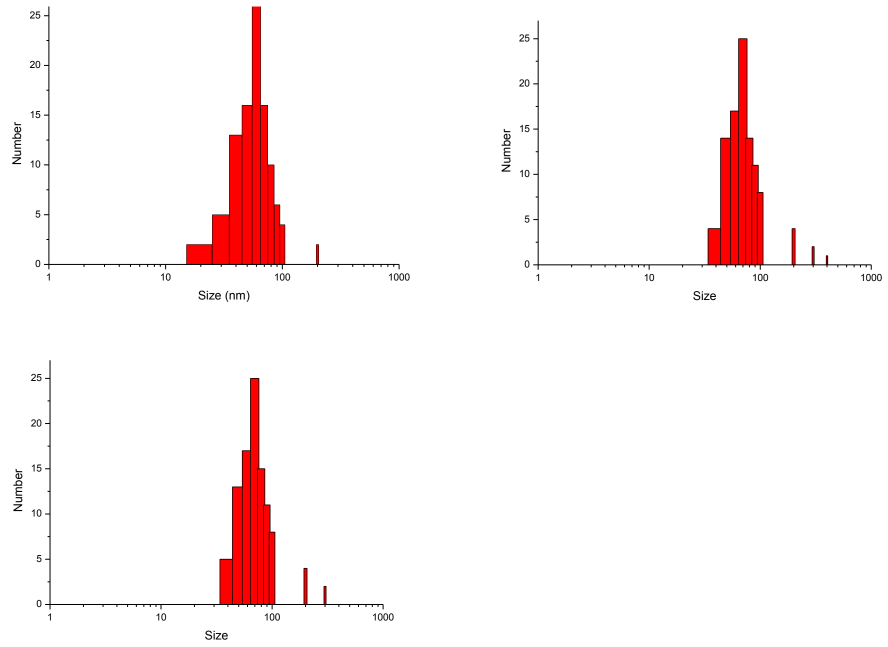


Fig.1 Five PELN particle size distribution

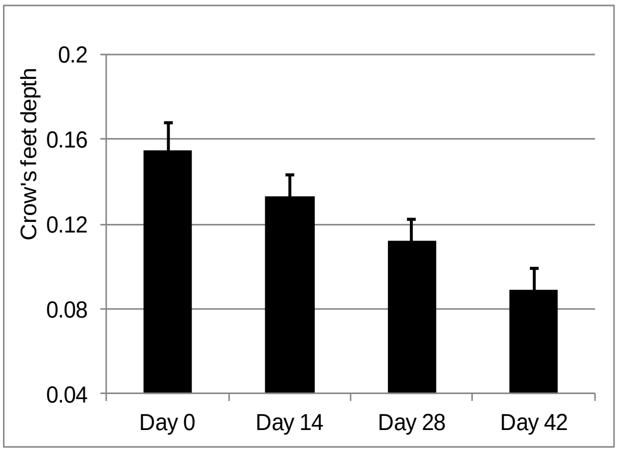


Fig. 2 Depth of crow's feet after use PELNs essence

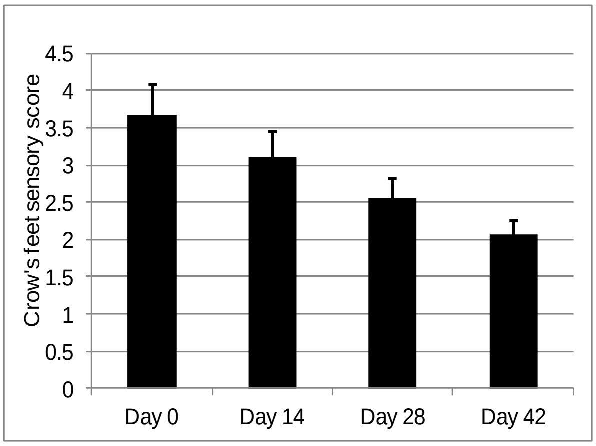


Fig. 3 Average sensory score of crow's feet after use PELNs essence

Twenty-six volunteers used the eye essence with PELN for three months. The testers measured wrinkle depth and evaluated wrinkles through sensory assessment. The experiment showed that crow's feet improved significantly after 42 days of use.

The average depth of crow’s feet was 0.155±0.013 mm on day 0, 0.133±0.010 mm on day 14, 0.112±0.010 mm on day 28, 0.089±0.010 mm on day 42, with 14.19%, 27.74%, 42.58% decrease of wrinkle depth on day 14, 28 and 42 (Fig. 2).

The average sensory score of crow’s feet was 3.68±0.40 on day 0, 3.11±0.34 on day 14, 2.55±0.26 on day 28, 2.06±0.19 on day 42, with 15.49%, 30.71%,44.02% decrease of sensory score on day 14, 28 and 42 (Fig. 3).

Fig.4 The improvement effect of removing the crow’s feet

From the before - and - after photos of the subjects using the eye essence with PELNs, it can be seen that the fine lines around the eyes became shallower and fewer, and even almost disappeared (Fig. 4). The skin around the eyes became more hydrated and elastic. The brightness of the eye - surrounding skin of some subjects also improved.

4. Discussion

PELNs have demonstrated promising potential in reducing crow's feet wrinkles through their multifunctional components. PELNs typically incorporate bioactive compounds such as polyphenols, carotenoids, and peptides, which exert anti-aging effects via distinct mechanisms. Antioxidant compounds in PELNs neutralize reactive oxygen species (ROS), mitigating oxidative stress-induced collagen degradation and extracellular matrix (ECM) disruption. Additionally, certain plant-derived peptides stimulate collagen synthesis, enhancing skin elasticity and reducing wrinkle depth. Hydrating agents, such as hyaluronic acid, further contribute to wrinkle reduction by improving skin hydration and barrier function.

The nanocarrier system in PELNs enhances bioavailability and targeted delivery of these active ingredients, ensuring efficient penetration into the dermal layers. This synergistic action addresses both intrinsic and extrinsic aging pathways, offering a comprehensive anti-aging solution.

Looking ahead, PELNs hold significant promise for future applications in skincare. Advances in nanotechnology and plant extract optimization are expected to further enhance their efficacy and biocompatibility. The integration of sustainable sourcing and precision delivery systems could position PELNs as a cornerstone in next-generation anti-aging formulations, potentially expanding their use in medical aesthetics and personalized skincare regimens.

There are examples of using 5 plants of *Centella asiatica*, *Panax ginseng*, *Vaccinium myrtillus* *Camellia reticulata*, and *Avena sativa* in cosmetics, and good results have been achieved.

Centella asiatica root helps boost collagen production and has antioxidant properties to fight free radicals, thus reducing wrinkles [7]. *Panax ginseng* root stimulates fibroblast activity for more collagen and elastin, enhancing skin elasticity [8]. *Vaccinium myrtillus* fruit is rich in antioxidants like anthocyanins, which protect skin from oxidative stress and prevent aging [9]. *Camellia reticulata* flower has antioxidants and anti - inflammatory compounds that keep skin healthy and reduce aging signs [10]. *Avena sativa* germ is a moisturizer and anti - inflammatory agent that keeps skin hydrated and less prone to wrinkles.

In this study, we used the PELNs of these five plants to replace regular plant extracts. Compared to the latter, PELNs have better biological activity and stronger penetration and absorption rates, which helps bring out their antioxidant, anti - inflammatory and anti - aging effects.

5. Conclusion

Herbal extracts, such as: *Centella asiatica*, *Panax ginseng* were already used in eye essence for anti crow's feet. In this study we extracted and purified PELNs from 5 herbs' different parts, particle size and zeta potential characterizes the basic parameters of PELNs. The PELN has bilayer and can keep ingredients active, which promote osmotic absorption and utilization. *Centella asiatica* exosome has regenerative and anti-wrinkle effects on the skin,which can activate genes related to skin aging.Exosomes from ginseng root have been shown to have anti-aging and skin-protective effects. We used *Centella asiatica*, ginseng and other three PELNs in eye essence, human skin test showed it can significantly improve crow's feet.

6. References

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