Development of Exosomal Raw Material from Upcycled Purslane (*Portulaca oleracea)* Waste Obtained by Green Chemistry Using Artificial Intelligence (AI) Modeling for Cosmetic Products

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ABSTRACT

Backround: Exosomes are nano-sized extracellular vesicles containing proteins, lipids, enzymes, and other substances. Recently, plant-derived exosomes have attracted attention as cosmeceutical materials due to their beneficial effects on human skin, including anti-aging, moisturizing, whitening, and regeneration properties. Upcycling involves converting by-products, waste materials, or discarded objects into new materials or products. Food and beverage waste is a primary source of materials for beauty products, especially natural and organic cosmetics, as these often contain food-grade ingredients rich in beneficial properties for the skin. Purslane extract offers anti-inflammatory, antioxidant, anti-aging, and moisturizing benefits, addressing various skin issues such as wrinkles and other signs of aging. Artificial neural networks, an application of artificial intelligence, are computing technologies that mimic the human brain's structure. They analyze existing data and generate new information using various learning algorithms. **Method:** After hot-air drying the leaf part of *Portulaca oleracea* (Purslane) waste at 45 °C for 24 h, according to green chemistry method using artificial intelligance modelling the purslane extract was obtained at 80°C for 3 h and was filtered through a 0.45

μm mesh filter. To obtain the exosomes ultracentrifugation method was used at 150,000 ×*g*, 4°C for 70 min. To conduct the morphological analysis of exosomes, SEM and TEM were performed. Size and zeta potential were measured. To measure the number of exosome particles per ml nanoparticle tracking assay (NTA) was performed. The exosomes was applied to human mesenchymal stem cells as a healthy tissue control to analyze the cell proliferation activity by Cell Titer Glo (Promega). Fish embryo toxicity test was performed to assess the effects of the exosomes on zebrafish embryonic development. **Results:** The morphology of exosomes isolated from purslane waste extract optimized by AI was analyzed through scanning and transmission electron microscopy. The average size of exosomes was approximately 122 nm. It was found that the total number of exosome was $2.42x10^{10}$ +/- $5.80x10^{8}$ particles/ml. The zeta potential of exosomes was -13 mV. The results were analyzed using the zeta sizer device, and it was detected that both the zeta potential of the exosomes and the stability of the working environment were rated as good. The exosomes showed statistically significant proliferative effects on human mesenchymal stem cells. Zebrafish embryos exposed to the dilutions of exosomes below the letal concentration exhibited development similar to the control. **Discussion and Conclusion**: Considering tissue regeneration capacities of hMSCs, repopulating of stem cells using plant derived exosomes can enable us to yield functional products, especially for longevity and anti-aging processes. These findings may lead to further approaching the use of AI modeling to obtain upcycled plant extracts for exosome derivation. This may open up new avenues to serve novel ingredients for cosmetics industry.

Key words: Plant Exosome, Artificial Intelligance (AI), Upcycle, Purslane extract, Cosmetics

1. INTRODUCTION

The cosmetics industry has recently taken notice of exosomes [1-3]. When incorporated into topical creams, serums, and masks, exosomes have demonstrated a range of medicinal and anti-aging benefits. These tiny vesicles, rich in proteins, lipids, and other compounds, support skin care by promoting healing and protection. They aid in boosting collagen production, reducing inflammation, and shielding the skin from external stressors. Additionally, exosomes can enhance the effectiveness of other active ingredients like hyaluronic acid, peptides, and antioxidants. [2].

Exosomes are small vesicles derived from endosomes that have gained significant attention over the past decade. First discovered in the extracellular space in the late 1980s, exosomes are

substances secreted by most living cells. These biologically small membrane-bound liposomes range in size from 30-500 nm. Vesicles are categorized based on their cell of origin, functions, and sizes. Exosomes are packed with DNA, RNA, proteins, lipids, enzymes, and other molecules. Research has shown that they contain cytokines, transcription factor receptors, and various other bioactive substances. [4-7].

Plant-derived exosomes, like their mammalian counterparts, contain a variety of components such as proteins, lipids, mRNA, microRNA, and unique bioactive substances specific to plants. These exosomes play a crucial regulatory role in the natural immune systems of plants and facilitate cell-to-cell communication. Due to their abundant availability, biocompatibility, and biodegradability, plant-derived exosomes show significant promise as cell-free therapies for various diseases. [8-13].

Purslane extract offers anti-inflammatory, antioxidant, anti-aging, and moisturizing benefits. It is abundant in minerals such as potassium, magnesium, calcium, and phosphorus, along with omega-3 and omega-6 fatty acids, and vitamins A, C, and E. Additionally, it contains the "super antioxidant" glutathione. Widely used as a herbal medicine in many countries, purslane possesses pharmacological properties including analgesic, anti-bacterial, skeletal muscle-relaxant, wound-healing, anti-inflammatory, and antioxidant activities. The antioxidants in purslane are effective in treating various skin issues, including wrinkles and other signs of aging. [14-16].

Artificial neural networks, a key application of artificial intelligence, are computing technologies that mimic the functioning of the human brain. They analyze existing data and generate new information using various learning algorithms [17]. Pythia® is a computer program that operates similarly to neural networks in the human brain. It identifies the most suitable neural network based on the available data and functions accordingly. The program features multiple layers with varying numbers of neurons at each level, which interact with one another akin to the neural structure in the brain. Pythia's specialized subprogram determines the number of layers and neurons. It employs the least squares method for training and developing the artificial neural network model. Upon completing the training, Pythia calculates the equations and functions used in each neuron to generate outputs from experiments based on the given inputs. It predicts potential outcomes for new input values, eliminating the need for further experiments. The advantage of artificial neural network modeling lies in its learning capability, enabling the examination of parameter effects and the generation of highly realistic results for unknown inputs [18].

Stem cells can self-renew themselves and differentiate into multi-lineage cells. Human mesenchymal stem cells (hMSCs) are the non-haematopoietic, multipotent stem cells that can differentiate into osteocytes, adipocytes and chondrocytes, neurocytes, and hepatocytes [19]. They are involved in tissue regeneration since they can differentiate into the appropriate cells at the site of injury [20]. What's more, they can be easily isolated from several bodily sources such as bone marrow, adipose tissue and dental pulp. Therefore, hMSCs are utilizied for several therapeutic strategies including regeneration of damaged tissues. Taken together, in this study we used human mesenchymal stem cells as a healthy cellular model (already exist in human body) considering their unique feature in tissue repair.

Here, we summarize the purification method, characterization and activity of plant exosomes derived from purslane waste extract optimized by artificial intelligance (AI) with a view to furthering the research and industrialization process of plant derived exosomes in the field of cosmetics.

2. MATERIALS AND METHODS

2.1 Purslane Waste Extraction Optimized by Artificial Intelligance (AI)

Based on different extraction conditions (temperature, solid-liquid ratio, duration, solvent type), the parameters to be used in developing extracts with the total flavonoid content via artificial neural network modeling have been determined through literature studies. After collecting the data, all values were entered into the Excel program. All parameters were accepted as inputs, and the total amount of flavonoids obtained from each extraction study was entered. The Pythia® computer program accepts copy/paste operations from Excel columns. After copying the inputs and outputs, the future function called "Evolutionary Optimization" in the Pythia program was selected and the program was run. Finally, the program provided the best neural network model showing the number of neurons in the layer, taking into account the smallest sum of the squares of deviations from the actual data. The program was trained to use a specific neural model for the actual data. After the training, the program's calculations for predictions were taken into account. These predicted values were then compared with the actual data.

2.2 Exosome Isolation by Ultracentrifugation

Purslane waste extract that optimized by AI were centrifuged three times at 4°C to remove cell debris, fibers, and large particles (1st centrifugation: 1,000 g for 10 mins, 2nd centrifugation: 3,000 g for 20 mins, 3rd centrifugation: 10,000 g for 60 mins). After the final centrifugation, supernatants were

collected and transferred to ultracentrifuge tubes. Then, the volume was brought to 30 ml with sterile PBS. To isolate exosomes, SW32 Ti rotor, OptimaXE-100 (Beckman Coulter, USA) was used and samples were centrifuged at 135,000 g for 70 minutes at +4 °C.

2.3 Characterization of the Purslane Derived Exosome

2.3.1 Electron Microscopy

2.3.1.1 Imaging of Exosomes by Scanning Electron Microscopy

A drop of the Purslane extract exosome suspension was deposited for overnight drying and imaged the following day in a Gemini SEM 360 SEM microscope (ZEISS) after sputter-coating the samples with gold.

2.3.1.2 Imaging of Exosomes by Transmission Electron Microscopy

For TEM analysis, the sample was incubated for 10 minutes by dropping it onto a formvar/carbon supported copper grid (Ted Pella 0180 formvar/Carbon 200mesh copper grid). At the end of the incubation period, without allowing the sample to dry, the grid was placed on 25 µL of 2% Uranyl acetate alternative solution (Ted Pella 19485 Uranyl acetate altetnative (Gadolinium acetate tetrahydrate)) on parafilm for 1 minute for staining. After the staining period, the sample-loaded grid was left to dry at room temperature and then examined with a Jeol JEM-2100Plus transmission electron microscope at an accelerating voltage of 200 kV.

2.3.2 Nanoparticle Tracking Assay (NTA)

NTA measurements were performed with a NanoSight NS300. The system consists of a camera system, a syringe pump, and a computer system to process the obtained data. The software used for capturing and analyzing the data was the NTA 3.4 Build 3.4.003. The laser beam was passed through the sample chamber, and the scattered light from the particles in the suspension was observed by a video camera placed in its path. All measurements were performed at 21.9 °C. The mean size and SD values obtained by the NTA software.

2.3.3 Particle Size and Zeta Potential Measurement

Particle size and Zeta potential of the samples suspended in particle-free water were measured with the Zetasizer Ultra (Malvern Panalytical) device (Exosomes; refractive index (n =1.38); absorption (k = 0.01). The ZP of Purslane extract exosome was measured thrice at 25 °C.

2.4 Cell Proliferation Assay

Human mesenchymal Stem Cells (UE7T-13 cells, no. RBRC-RCB2161; RIKEN, Japan) were plated at 5000 cells/well into 96 black well plates (3603, Corning) and cultured in DMEM, low glucose (Gibco) containing 10% FBS at 37 °C in 5% CO_2. Cells were cultured overnight and exosomes were added to the cells at diluted concentrations. The cells were incubated under standard culture conditions for 24 hr. To measure the activity of dehydrogenases, which increases in parallel with the increase in the number of living cells, cell proliferation was measured using Cell Titer Glo (Promega) according to the manufacturer's instructions. The values obtained from the exosome-treated cells were normalized against the signal for DMEM-only (control) treated cells.

2.5 Zebra Fish Embryo Toxicity Test

Male and female (2:1) zebrafish were kept in an aquarium rack system at 28°C under a 14/10 light/dark cycle.Fertilized eggs were collected and embryos were rinsed under water several times before used. Embryos were exposed to diluted solutions of exosome. E3 Medium solution was placed in well plates for the control group. Each exposure group was prepared as triplicates in 24-well plates having 20 embryos in each of them. They were monitored to evaluate their development and the images of malformations were recorded by using a stereomicroscope (Zeiss Discovery V8) for 72 hours post-fertilisation (hpf). Hatching rate is defined as the ratio of hatched embryos to the whole number of alive embryos in each well. Accordingly, hatching and mortality analyses were carried out every 24 h.

3. RESULTS

The morphology of exosomes isolated from *P. oleracea* extract was analyzed through scanning and transmission electron microscopy (SEM, TEM).

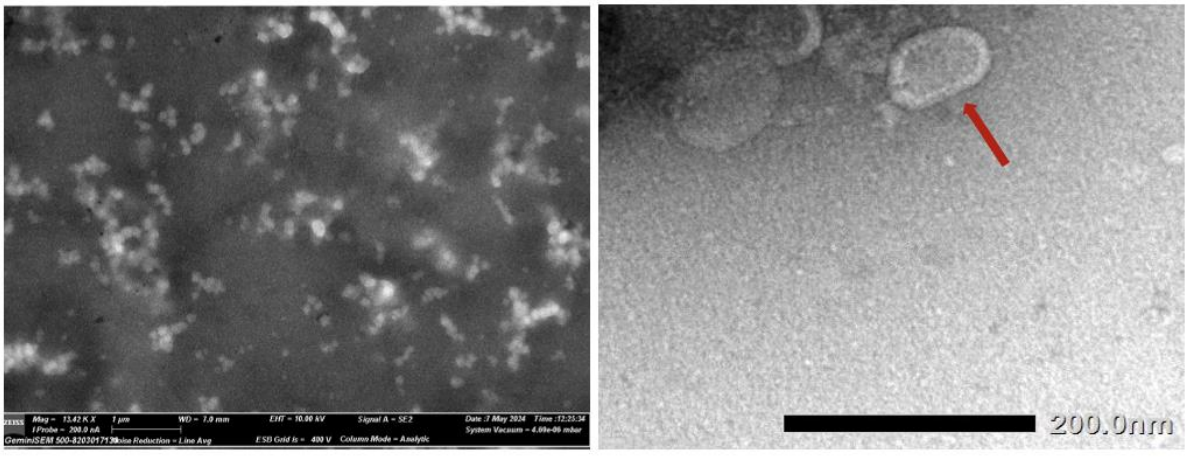

Figure 1: Morphology of purslane derived exosome was observed using SEM (left panel) and TEM (right panel). Scale bars are 200 nm for both the panels.

In order to investigate plant exosomes, we conducted nanoparticle tracking analysis that allows us to measure the number of exosome particles per ml. Nanoparticle-tracking analysis showed that the average size of the purslane derived exosome was approximately 122 nm (92-297 nm) and the total number of exosome was $2.42x10^{10}$ +/- $5.80x10^{8}$ particles/ml (Figure 2).

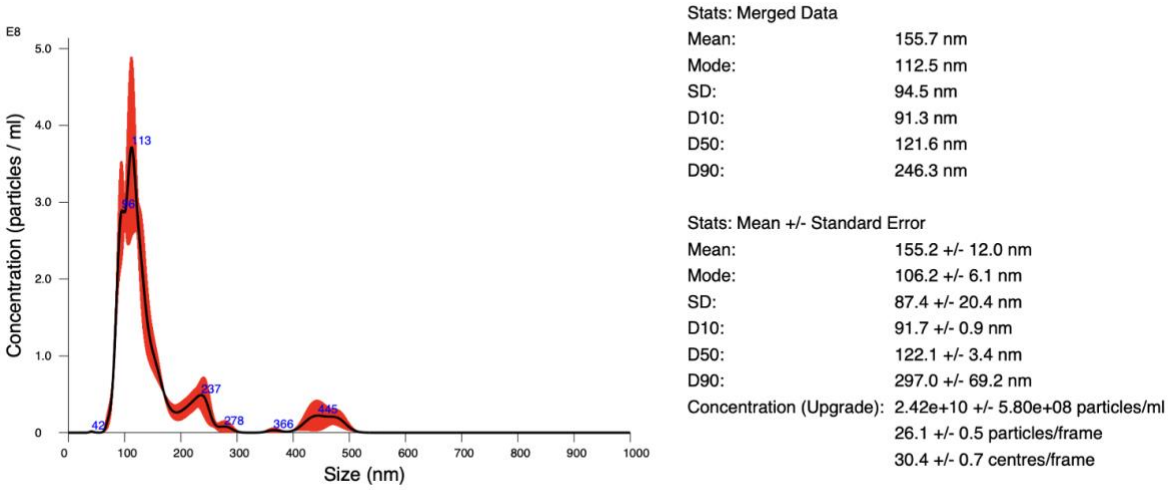

Figure 2. Determination of the size and concentration of purslane derived exosome: dilution factor—1:100; laser—red laser; camera level—16; detection threshold—18; slider shutter—1300; slider gain—295.

To further investigate the characteristics of purslane derived exosomes, the size distribution and zeta potential of the exosomes was measured and zeta potential of the exosomes was found -13 mV. The results were analyzed using the zeta sizer device, and it was detected that both the zeta potential of the exosomes and the stability of the working environment were rated as good (Figure 3).

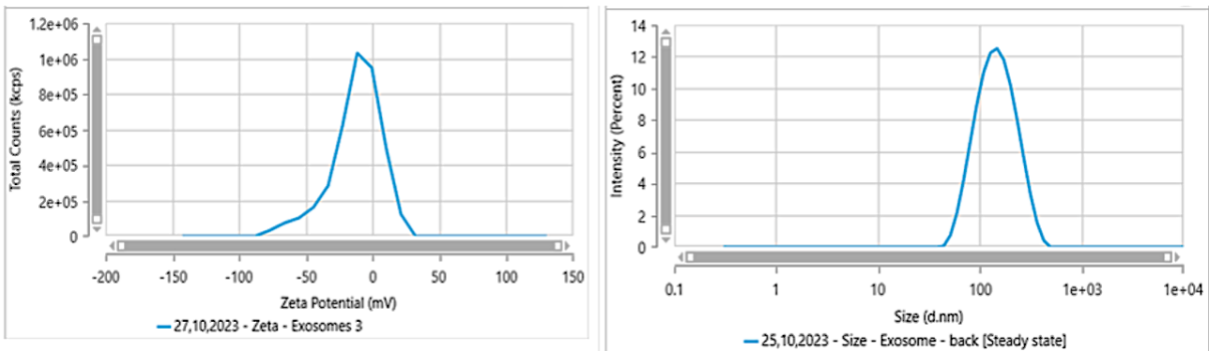

Figure 3: Zeta potential and size distributions of purslane derived exosomes.

The exosomes showed statistically significant proliferative effects on human mesenchymal stem cells (hMSCs). Cell viability and cell morphology increased 20% when the number of exosomes was 0,6E+08 (particles/ml) (Figure 4).

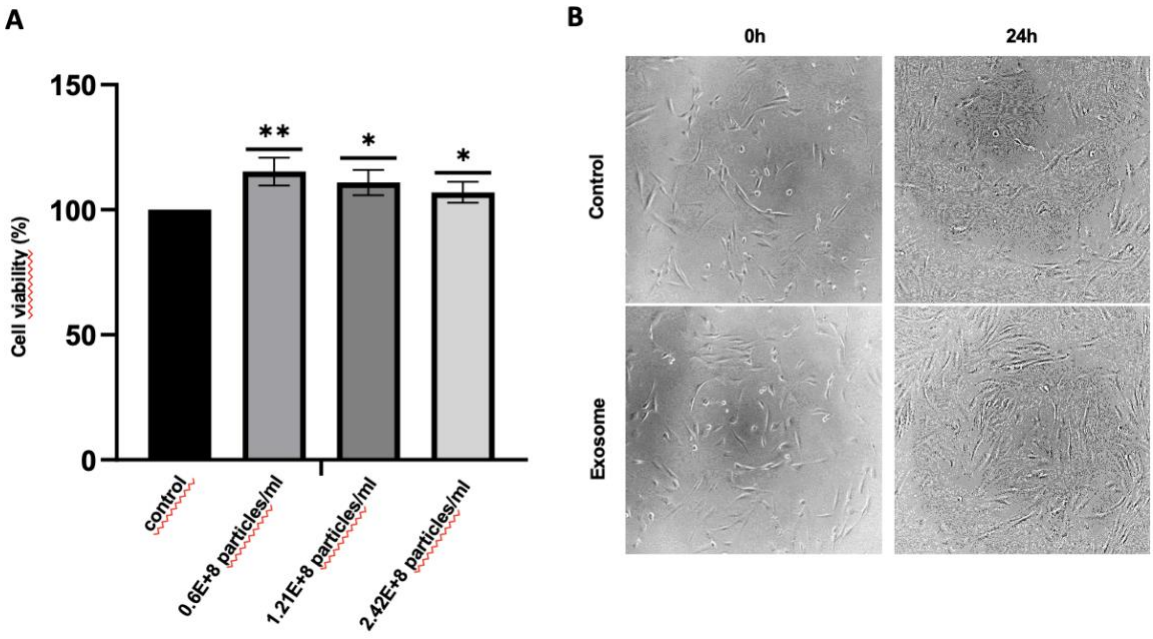

Figure 4: A % Cell viability graph of the exosomes against hMSCs after 24 hours of exposure at the concentration ranging from 0,6E+8 to 2,42E+8 particles/ml. Statistical significance was determined by student's t test ($P < 0.05$: *, $P < 0.01$: **). **B** Morphological changes in exosome treated (0,6E+08) or untreated (control) human mesenchymal stem cells.

Zebrafish embryos exposed to dilutions of exosomes below the letal concentration exhibited development similar to the control (Figure 5).

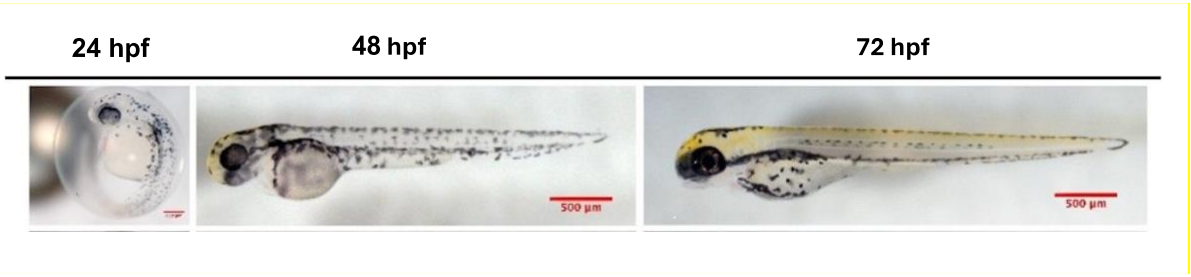

Figure 5: Representative images of zebrafish embryos with similar development to the control group at 24, 48 and 72 hours post-fertilization (hpf). Scale bar: 500 µm.

DISCUSSION

Exosomes carry a range of bioactive compounds that regulate cell communication and growth. Increasing evidence points to the therapeutic effects and health benefits of plant-derived exosomes. For instance, exosomes from ginseng inhibit melanoma growth. Additionally, exosomes from grapefruit, carrot and ginger inhibit inflammation [10]. Similarly, beta vulgaris exosomes show antioxidant activities and inhibition of fibroblast migration to prevent scar formation and exert anti-aging effects [12]. In addition, citrus lemon derived exosomes exhibit anti-cancer effects by decreasing the expression of anti-apoptotic genes [21]. Portulaca oleracea is a medicinal herb widely utilized in many countries. Various studies have emphasized the extensive pharmacological and cosmetics effects of purslane, such as its anti-inflammatory, anti-aging, antioxidant and antitumor properties. In addition, orally administered purslane derived exosome like nanoparticles may have therapeutic effect on ulcerative colitis [22, 23]. These evidences suggest the potential applications of plant derived exosomes in the pharmaceutical and cosmetic industries.

Most recently, artificial intelligence (AI) has been a promising tool to optimize a wide range of protocols for industrial use including cosmetics and health care. AI technology provides opportunities for product innovation and development [24]. Moreover, AI can drive innovation in the cosmetics raw material industry, allowing businesses to create new and unique products that meet the ever-evolving demands of their clients.

Accordingly, in this study, we used AI optimized purslane waste extract collection to obtain exosomes with high quality and quantity. The first set of experiments aimed to collect, identify and quantify the exosomes using AI optimized plant extraction protocol. According to our results, the morphology and size of the purslane derived exosomes determined by both scanning electron microscopy (SEM) and transmission electron microscopy (TEM) confirmed the overall spherical shape of the exosomes. Beside that, the mean diameter of the plant derived exosomes was correlated with NTA and size distribution data. Secondly, we tested functionality of the exosomes on human stem cells to further adopt its potential use as a proliferative agent in cosmetic products. The exosomes are found optimum when used between $2{,}42 \times 10^8$-$0{,}6 \times 10^8$ (particles/ml) to induce proliferation of the cells. Critically, ideal use of exosomes require optimum doses rather than high concentrations. Toxicity of the material is another issue to be solved. For this reason, we applied for zebrafish embryo toxicity test to double check the cytotoxic effects. Our findings demonstrated similar outcomes with *in vitro* cell viability analyses indicating the safety use of the obtained exosomes.

CONCLUSION

To the best of our knowledge, by this study, for the first time, we showed the use of AI modelling to extract purslane waste for exosomal derivation. Our results revealed that extracted exosomes using AI based technology provides high-purity and high-stability to further use in cosmetic industry.

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CONFLICT OF INTEREST STATEMENT

The authors report no conflicts of interest in this work.

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