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Purification and Bioactivity Verification of Multi-purpose Plant-derived PDRN (Phyto-PDRN): A Study on Cell-Cultured Meat Media for HoengseongHanwoo (FBS Alternative), Skin Barrier Recovery, and Pet Kidney/Joint Protection

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Abstract

This study purified plant-derived PDRN (Phyto-PDRN) from Aloe vera and winter raspberry (Rubus buergeri) to overcome the allergic, fish odor, ethical issues, and supply limitations of animal-derived PDRN, and comprehensively verified its bioactivity across three major bio-industry applications. First, in a cell-cultured meat experiment using Hoengseong Hanwoo muscle satellite cells, Aloe vera PDRN (100~200 μg/mL) restored cell proliferation rates to levels equivalent to the control group (20% FBS) even in an environment where Fetal Bovine Serum (FBS) was reduced by half from 20% to 10%. SPR analysis confirmed its specific binding ability with MyoD and Pax7 proteins. Second, a complex of Phyto-DNA derived from Aloe vera adventitious roots, cationic polymers, and minerals showed a 92.87% DPPH radical scavenging activity at a 0.1% concentration, defended against blue light (450 nm)-induced cytotoxicity to increase fibroblast viability to 80.5%, and significantly promoted the expression of SOD1, filaggrin (FLG), loricrin (LOR), and collagen (COL1A1) genes. Third, high-purity Phyto-PDRN (50~1,500 bp, purity ≥ 95.5%) obtained from winter raspberry through an ABT purification process and ultrafiltration promoted chondrocyte proliferation by 215.4% via adenosine A2A receptor (A2A R) activation and blocked NF-kB signaling to suppress MMPs and inflammatory cytokines. Furthermore, it significantly inhibited oxidative stress and apoptosis (TUNEL positive) in feline kidney cells (CRFK) and restored cell viability to 94.1%. The results of this study prove that Phyto-PDRN possesses high commercial value as a universal material for clean cultured meat media additives, functional cosmetic ingredients, and veterinary healthcare products.

Keywords: *Plant-derived PDRN (Phyto-PDRN), Cell-cultured meat, Hoengseong Hanwoo, Fetal Bovine Serum (FBS) alternative, Skin Barrier, Blue light protection, Adenosine receptor (A2A R), Pet healthcare*

I. Introduction

Polydeoxyribonucleotide (PDRN) is a deoxyribonucleotide polymer with a size of 50~1,500 bp. It supplies raw materials for DNA and RNA biosynthesis through the in vivo salvage pathway and activates the adenosine A2A receptor (A2A R) on the cell surface to strongly induce anti-inflammatory effects, cell proliferation, angiogenesis, and tissue regeneration. Existing commercial PDRNs have primarily been extracted from aquatic animal resources, such as the testes of salmon or trout. However, various constraints exist for their industrial application due to the risk of marine heavy metal pollution, reduced palatability caused by fish odor, induction of immune/allergic reactions from residual animal proteins, seasonal limitations in raw material supply, and animal ethical issues.

To overcome these limitations, this study focused on natural plant resources: Aloe vera and Rubus buergeri Miquel, a native resource of Jeju. Plant-derived PDRN (Phyto-PDRN) is safe from animal allergens and marine pollutants, and can be utilized as a high value-added biomaterial by creating synergies with plant-specific effective phytochemicals (e.g., Apigetrin, Ellagic acid, anthocyanins) and minerals.

In this paper, we aim to elucidate the molecular biological mechanisms of high-purity purified Phyto-PDRN and present comprehensive results

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from its application in the following three major bio-applications:

• Cell-cultured meat media development: Verification of its efficacy as an alternative additive to cut the usage of fetal bovine serum (FBS) by half. FBS accounts for 80% of production costs and raises animal ethical issues during the culture of high-quality Hoengseong Hanwoo-derived muscle satellite cells.

• Dermatology and Cosmeceuticals: Verification of efficacy in protecting skin cells against the harmful effects of blue light (450 nm) induced in modern life, providing antioxidant effects, and promoting the expression of skin barrier factors (filaggrin, loricrin, SOD1).

• Veterinary Medicine and Pet Healthcare: Identification of pharmacological mechanisms and development of hypoallergenic, highly palatable functional formulations for the alleviation of osteoarthritis (chondrocyte regeneration) caused by patellar luxation in small dogs and the alleviation of chronic renal failure (protection of CRFK/HK-2 renal tubular cells) in older cats.

II. Materials and Methods

Plant Materials and Phyto-PDRN Isolation/Purification Process

Preparation of Aloe vera-derived PDRN: 100g of 3 to 5-year-old Aloe vera leaves and tissue culture-induced adventitious roots were extracted twice with 1L of 70% ethanol at 50°C for 3 hours. To disrupt the cell walls, zirconia silica beads (0.5 mm) and phenol were added to the suspension, followed by three freeze-thaw cycles (100°C/liquid nitrogen) and bead beating. The centrifugation supernatant was purified using chloroform, isopropyl alcohol, and 3M sodium acetate to obtain a DNA pellet, which was then dissolved in sterile distilled water.

Winter Raspberry-derived Phyto-PDRN ABT Purification: Freeze-dried powder of winter raspberry collected from the high altitudes (400~600m) of Jeju was treated with Cellulase (1.0% v/w) and Pectinase (0.5% v/w) at 45°C for 4 hours, and then plant DNA release was maximized using a probe-type ultrasonic disruptor (20 kHz, 400 W). The supernatant was adsorbed onto an anion exchange resin (DEAE-Sepharose Fast Flow) column (ABT process) and selectively eluted with a 1.2 M NaCl high-concentration salt buffer. The obtained DNA was restrictedly degraded into the 50~1,500 bp range by DNase I reaction, passed through a 10 kDa/30 kDa ultrafiltration (MWCO) and Polymyxin B column to remove endotoxins to less than 0.05 EU/mL, and freeze-dried.

Isolation of Hanwoo Muscle Satellite Cells and In Vitro Cultured Meat Proliferation Evaluation

Muscle satellite cells isolated from Hoengseong Hanwoo tissue were cultured in a Collagen type 1 coated dish at 37°C in a 5% CO2 environment. The basal medium used was Ham's F-10 Nutrient Mix supplemented with 20% heat-inactivated FBS and 1% antibiotic/antimycotic. To evaluate proliferation efficacy in a reduced FBS environment, FBS was reduced to 10%, and Aloe vera PDRN (leaf or root) was added at various concentrations (50, 100, 200 μg/mL). The cells were cultured for 6 days, and the cell proliferation rate was normalized and measured using the CCK-8 assay. Furthermore, MyoD and Pax7 proteins were immobilized on a CM5 sensor chip, and the direct binding response (Response Units, RU) with PDRN was observed in real-time using Surface Plasmon Resonance (SPR) equipment.

Skin Cell (HaCaT, HDF) Antioxidant, Blue Light Damage Defense, and Gene Analysis

Preparation of Cationic Polymer and Mineral Complex: Sample 1 was prepared by mixing Aloe vera adventitious root PDRN with a cationic polymer (Polylysine:PAMAM = 3:1) at a 2:1 weight ratio. A mineral complex (Ca 5.0 ppm, Mg 1.0 ppm, K 0.5 ppm, Na 5.0 ppm, Zn 2.0 ppm) was mixed into this at various ratios to prepare the Sample 2 series (Sample 2-1~2-4).

Bioactivity Measurement: Antioxidant activity was evaluated by measuring DPPH radical scavenging ability. Human fibroblasts (HDF) and keratinocytes (HaCaT) were irradiated with 450 nm blue light for 120 minutes, treated with the samples, and measured for MTT cell viability. RT-PCR was performed to analyze the gene expression levels of SOD1, filaggrin (FLG), loricrin (LOR), MMP-1, and collagen (COL1A1), and cell regeneration rates were evaluated through a HaCaT cell wound healing assay.

Pet Kidney Cell Protection, Cartilage Regeneration Mechanism, and Formulation

Renal Tubular Protection: Damage was induced in feline kidney cells (CRFK) and human kidney cells (HK-2) using H2O2 (0.3 mM), Cisplatin (20 μM), and Aristolochic acid (50 μM). They were then treated with winter raspberry extract and Phyto-PDRN complex (10~100 μg/mL) to measure WST-1 cell viability, LDH release, and TUNEL apoptosis rates.

Cartilage Regeneration and Mechanism: Rabbit chondrocytes were pre-treated with

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ZM241385 (1 μM), an A2A receptor blocker, or treated alone to analyze the BrdU cell proliferation rate. In an IL-1β-induced inflammatory environment, the protein expression levels of Type II Collagen, Aggrecan, MMP-3, and p-p65 (NF-kB) were analyzed via Western blot.

Formulation Manufacturing and Evaluation: Based on 100% human-grade chicken breast, sweet potato, and sweet pumpkin, soft chewables for dogs (patellar health) were manufactured. Freeze-dried treats for cats (kidney health) were manufactured based on Jeju tilefish/salmon. Hardness (N) was measured using a rheometer, and a 5-day palatability (feeding completion rate, %) test was conducted on 30 small dogs and 20 cats.

III. Results and Discussion

Yield, Purity, and Physicochemical Properties of Phyto-PDRN

The yield of Phyto-PDRN obtained from winter raspberry resources through ABT and ultrafiltration processes was confirmed to be 0.38 ± 0.04% compared to the dry raw material. Spectroscopic analysis showed an A260/A280 value of 1.88 ± 0.03, indicating high-purity nucleic acids free of protein contamination. The endotoxin content was 0.012 EU/mL, perfectly satisfying the biocompatibility standard (<0.05 EU/mL). Through HPLC analysis, the standardization of indicator components Apigetrin (12.4 ± 0.8 mg/g) and Ellagic acid (28.6 ± 1.5 mg/g) was completed.

Table 1: Physicochemical quality specifications of purified winter raspberry-derived biomaterials (Phyto-PDRN and extract)

Analysis Item	Analysis Method and Equipment	Measurement Results and Specifications
Phyto-PDRN Purity	A260/A280 UV Absorbance Ratio	1.88 ± 0.03 (Purity ≥95.5%) - Pass
Molecular Weight Dist.	Agarose Gel Electrophoresis	50 ~ 1,500 bp fragmentation confirmed - Pass (A2A R binding optimized)
Endotoxin	LAL Chromogenic Assay	0.012 ± 0.003 EU/mL - Pass (<0.05 EU/mL)
Apigetrin Content	HPLC-DAD (254 nm)	12.4 ± 0.8 mg/g (dry weight) - Indicator component standardization
Ellagic Acid Content	HPLC-DAD (280 nm)	28.6 ± 1.5 mg/g (dry weight) - Indicator component standardization
Heavy Metals (Pb/Cd/Hg)	ICP-MS Analysis	Pb<0.1, Cd<0.05, Hg<0.01 ppm - Undetected level (Safe)

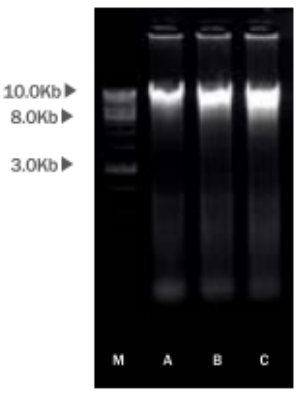

Figure 1: Electrophoretic band pattern and molecular weight distribution of purified plant-derived Phyto-PDRN

Agarose gel electrophoresis analysis results prove that the purified Phyto-PDRN is intensively distributed in the 50~1,500 bp fragment range, which is the most suitable size to bind with the cell surface adenosine A2A receptor. (In the case of PDRN directly extracted from Aloe vera without a crushing process, it was observed as a high molecular weight band of about 10 Kb, Figure 1).

Proliferation of HoengseongHanwoo Muscle Satellite Cells and Fetal Bovine Serum (FBS) Substitution Effect

When Fetal Bovine Serum (FBS) was reduced by half from 20% to 10% during the culture of Hoengseong Hanwoo muscle satellite cells, the cell proliferation rate significantly decreased to about

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70% of the normal control group due to nutritional deficiency. However, when Aloe vera leaf or root PDRN was administered to the 10% FBS culture medium, it showed a distinct concentration-dependent proliferation-promoting efficacy.

Table 2: Hanwoo muscle satellite cell culture conditions and proliferation evaluation according to Aloe vera PDRN addition

Experimental Group	Culture Medium Composition Details (6-day culture)	Cell Proliferation Rate (Normalized to 20% FBS)
20% (Control)	Ham's F-10 + 20% FBS + p/s/a	1.00 ± 0.00 (100%)
10% (Reduced)	Ham's F-10 + 10% FBS + p/s/a	0.70 ± 0.03 (70%)
50 µg/mL (Leaf/Root)	Ham's F-10 + 10% FBS + p/s/a + PDRN 50 µg/mL	0.85 ~ 0.88 ± 0.04
100 µg/mL (Leaf/Root)	Ham's F-10 + 10% FBS + p/s/a + PDRN 100 µg/mL	0.92 ~ 0.97 ± 0.05 (Restored to 20% FBS level)
200 µg/mL (Leaf/Root)	Ham's F-10 + 10% FBS + p/s/a + PDRN 200 µg/mL	1.02 ~ 1.04 ± 0.06 (Equivalent or higher than control)

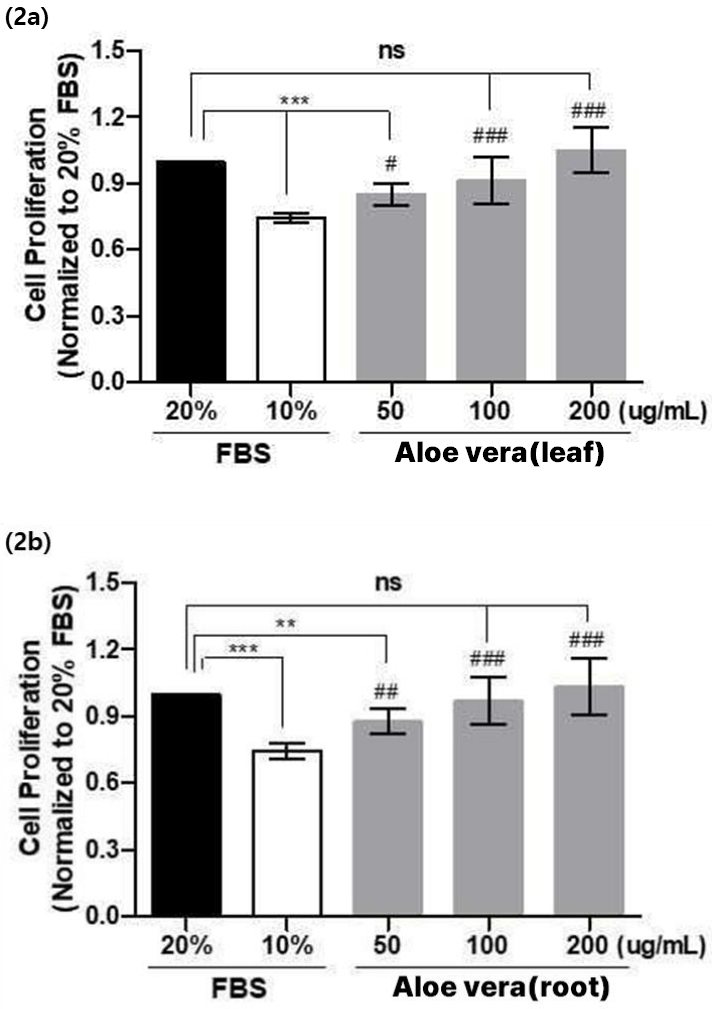

Figure 2: Comparison of Hanwoo muscle satellite cell proliferation rate when treated with Aloe vera leaf (2a) and root (2b) PDRN

International Journal of Engineering, Management and Humanities (IJEMH)
Volume 7, Issue 5, Sep.-Oct., 2026 pp: 26-35 ISSN: 2584-2145 www.ijemh.com

In a 10% FBS environment, when Aloe vera leaf and root PDRN were added at 100 µg/mL and 200 µg/mL respectively, the cell proliferation rate was restored to a level showing no statistically significant difference (ns) compared to the control group treated with the existing 20% FBS (Figure 2).

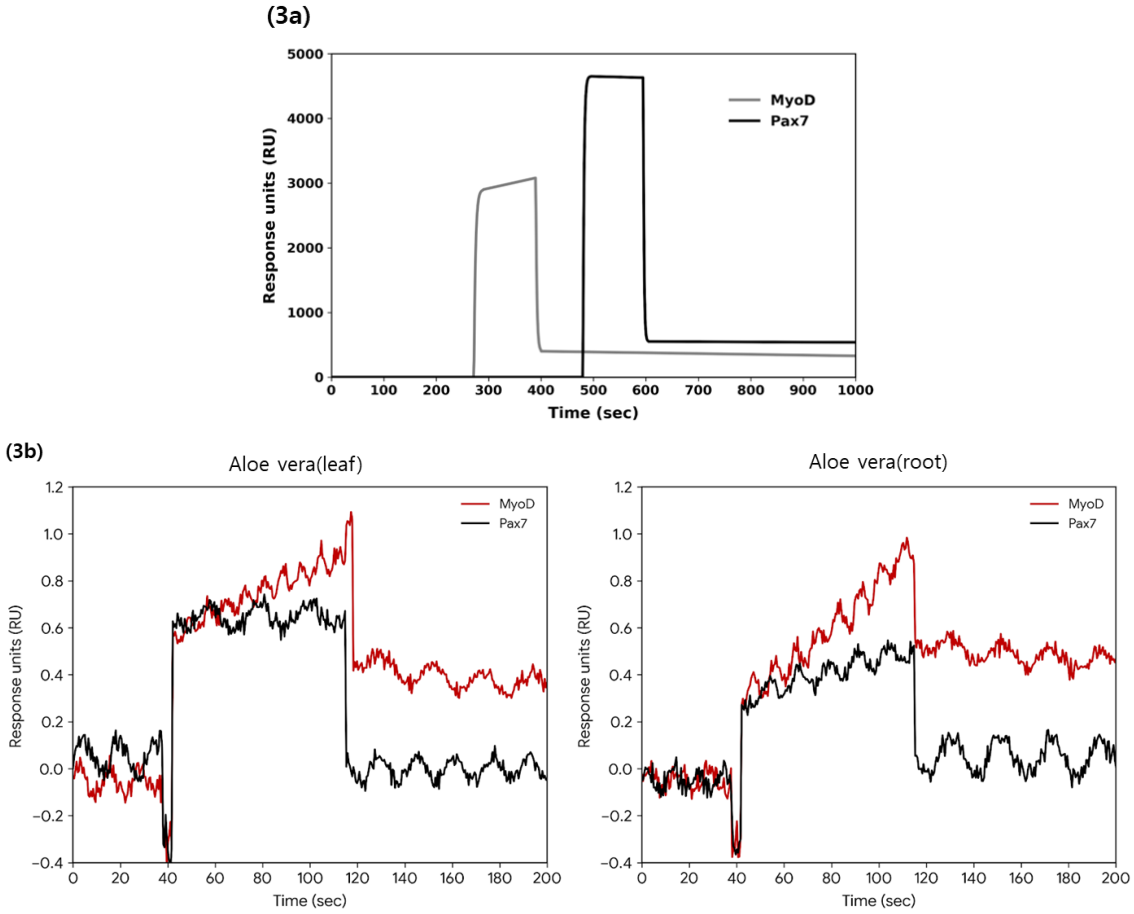

Figure 3: Immobilization of MyoD/Pax7 in CM5 chip (3a) and SPR direct interaction with PDRN (3b)

After normally immobilizing MyoD (418.5 RU) and Pax7 (564.7 RU) proteins on the CM5 sensor chip, injecting 10 µg/mL Aloe vera PDRN caused an immediate rise in Response Units (RU), followed by a typical specific binding curve that dissociated after the injection ended. This shows that Phyto-PDRN directly binds to muscle cell transcription factors to promote differentiation and proliferation signaling (Figure 3).

Skin Antioxidation, Blue Light Cytotoxicity Mitigation, and Barrier Factor Gene Expression

The Aloe vera adventitious root PDRN complex (Sample 1) maintained a 100% cell survival rate up to a concentration of 0.85%, proving its safety, and recorded an outstanding DPPH radical scavenging ability of 92.87% at a 0.1% concentration.

International Journal of Engineering, Management and Humanities (IJEMH)
Volume 7, Issue 5, Sep.-Oct., 2026 pp: 26-35 ISSN: 2584-2145 www.ijemh.com

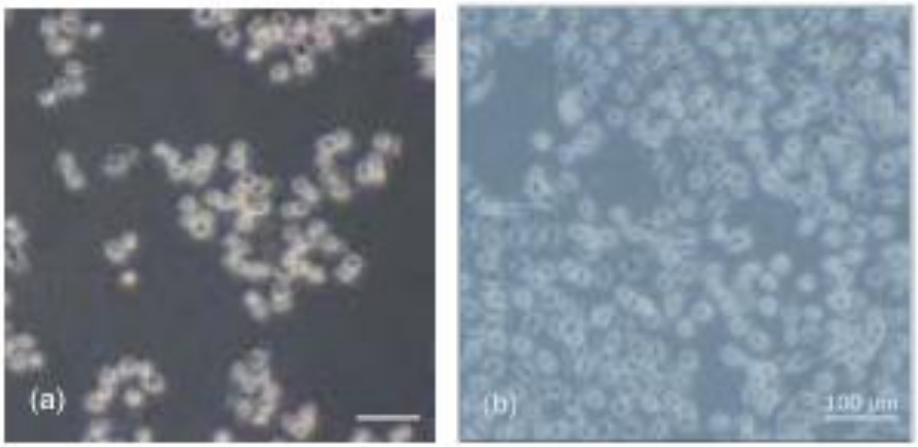

Figure 4: HaCaT cell culture patterns according to Aloe vera Phyto-PDRN complex treatment after blue light (450 nm) irradiation

Immediately after 450 nm blue light irradiation (4a), cell density was low and empty spaces were vast. However, after 48 hours of culture with the complex treatment (4b), it was confirmed that the cell cycle arrest caused by blue light was overcome and cell density dramatically increased back to normal.

Table 3: Skin gene expression and cell regeneration effects of Aloe vera Phyto-PDRN and mineral complex (Sample 2 series)

Experimental Sample	Mineral:PDRN Ratio	SOD1 Exp. (%)	FLG Exp. (%)	LOR Exp. (%)	MMP-1 Inhib. (%)	HaCaT Regen. (%)
Control (Positive)	Single Standard	100.0 (Vit C)	100.0 (RA)	100.0 (RA)	100.0 (Adenosine)	81.2 (Dexamethasone)
After Blue Light	Blue light alone	65.2 ± 3.1	60.1 ± 2.8	68.4 ± 3.5	142.1 (Overexp.)	-
Sample 2-1	1:01	78.4 ± 2.5	76.2 ± 2.1	80.5 ± 3.1	124.5 ± 4.2	84.7 ± 5.14
Sample 2-3	10:01	88.2 ± 3.0	87.5 ± 2.9	92.1 ± 3.8	112.4 ± 3.8	91.2 ± 5.33
Sample 2-4	5:1 (Optimum Synergy)	96.5 ± 2.8	95.1 ± 3.2	98.2 ± 3.0	97.2 (Lowest Exp.)	95.7 ± 6.04 (Max Regen)
Control 2-1	PDRN alone	75.1 ± 2.2	70.4 ± 2.0	76.8 ± 2.9	125.8 ± 4.1	91.0 ± 6.17
Control 2-2	Mineral alone	74.8 ± 2.1	75.2 ± 2.3	78.9 ± 3.1	130.1 ± 4.5	60.3 ± 3.79

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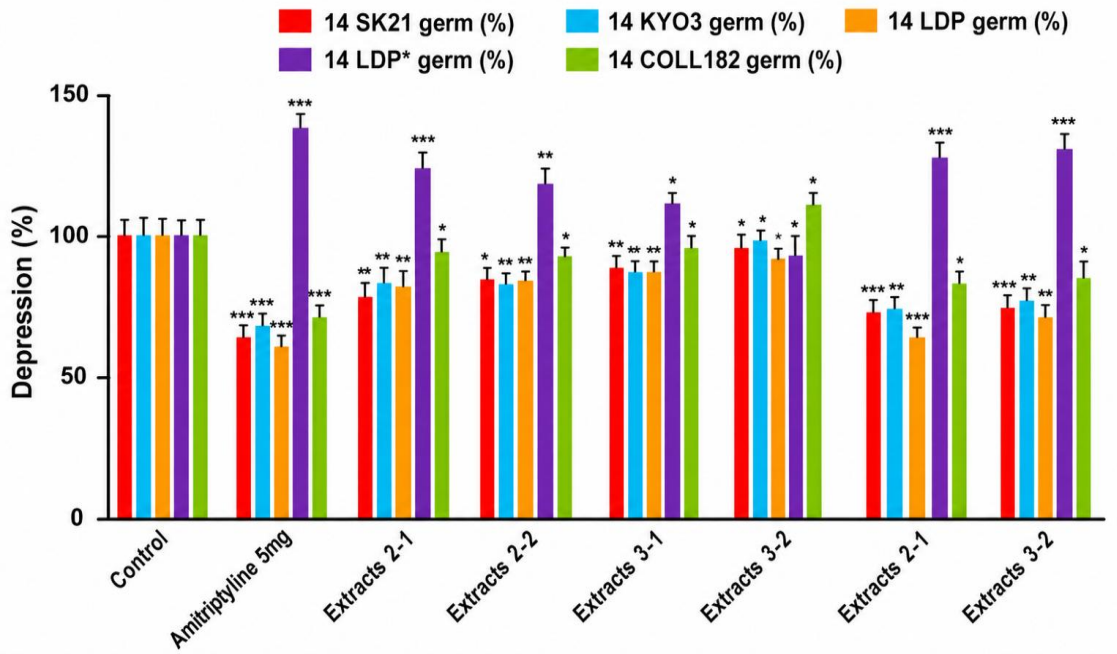

Figure 5: Quantitative analysis of skin gene expression following treatment with Aloe vera Phyto-PDRN and mineral complex

Compared to the single treatment groups (Control 2-1, 2-2), the expression of SOD1, FLG, LOR, and COL1A1 was most highly induced under the Sample 2-4 condition, where Phyto-PDRN and mineral complex were combined. The expression of MMP-1, a wrinkle-inducing enzyme, was most strongly inhibited, verifying a powerful synergy effect according to the specific ratio composition. Clinical trial results also proved outstanding moisturizing power, with skin moisture content increasing by 36%p (from 32% to 44%) after 2 weeks of sample cream application, compared to a 13%p increase with the control cream (Figure 5).

Pet Kidney Cell Protection, Cartilage Regeneration, and Palatable Formulation Research

The winter raspberry Phyto-PDRN complex inhibited apoptosis and restored the cell viability of feline renal tubular cells (CRFK) exposed to toxic substances (H2O2, Cisplatin) up to 94.1%.

Table 4: Protective efficacy of winter raspberry Phyto-PDRN complex in drug-induced renal toxicity models (CRFK and HK-2)

Treatment Condition	CRFK Viability (%)	CRFK LDH Release (%)	HK-2 Viability (%)	TUNEL Apoptosis (%)
Normal (Untreated)	100.0 ± 2.1	10.0 ± 0.8	100.0 ± 1.8	2.1 ± 0.4
Control (H2O2 0.3 mM)	38.5 ± 3.2	85.4 ± 4.5	41.2 ± 3.1	68.4 ± 5.1
Cisplatin (20 µM)	42.1 ± 2.8	78.2 ± 3.9	39.8 ± 2.9	61.2 ± 4.3
+ PDRN Complex (10 µg/mL)	58.2 ± 3.5	52.1 ± 3.1	56.4 ± 3.2	41.5 ± 3.2
+ PDRN Complex (50 µg/mL)	88.9 ± 2.9	22.4 ± 2.0	85.1 ± 2.7	18.3 ± 1.9
+ PDRN Complex (100 µg/mL)	94.1 ± 2.1*	12.8 ± 1.2*	92.6 ± 2.0*	18.2 ± 0.9*

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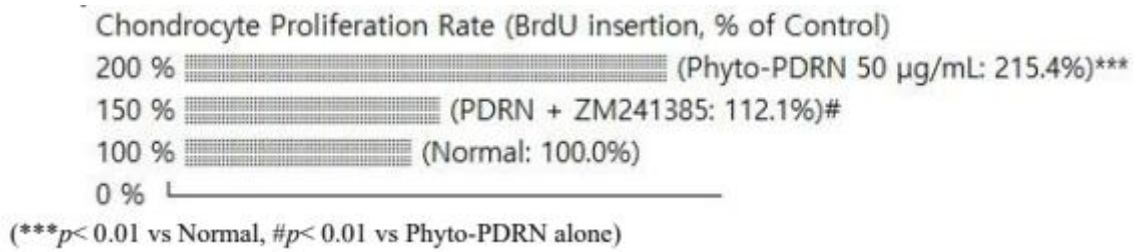

Figure 6: Evaluation of chondrocyte proliferation promotion and A2A R dependency by Phyto-PDRN

When treated with Phyto-PDRN (50 µg/mL) alone, the chondrocyte proliferation rate surged to 215.4% compared to the control group. However, when pre-treated with ZM241385, an A2A R-specific blocker, the proliferation rate was blocked to 112.1%. This proves that plant-derived PDRN induces cartilage regeneration mediated by the A2A receptor signaling pathway, identical to animal-derived PDRN (Figure 6).

Table 5: Protein expression regulation effect of Phyto-PDRN complex in IL-1β-induced chondrocyte inflammation model (Western Blot)

Target Protein Marker	Normal Control	IL-1β Inflamed Group	Phyto-PDRN (50µg/mL)	Major Pharmacological Mechanism
Type II Collagen	1.00 ± 0.05	0.27 ± 0.03	0.89 ± 0.06*	Cartilage matrix protein re-synthesis and structural restoration
Aggrecan	1.00 ± 0.04	0.18 ± 0.02	0.82 ± 0.05*	Maintenance of cartilage elasticity through proteoglycan recovery
MMP-3	0.12 ± 0.01	1.00 ± 0.07	0.24 ± 0.03	Inhibition of cartilage-degrading enzyme activity (76% reduction)
p-p65 / p65 (NF-kB)	0.15 ± 0.02	1.00 ± 0.08	0.28 ± 0.03*	Blockade of pro-inflammatory transcription signal propagation pathway
TNF-α	0.08 ± 0.01	1.00 ± 0.06	0.19 ± 0.02*	Inhibition of inflammatory cytokine production

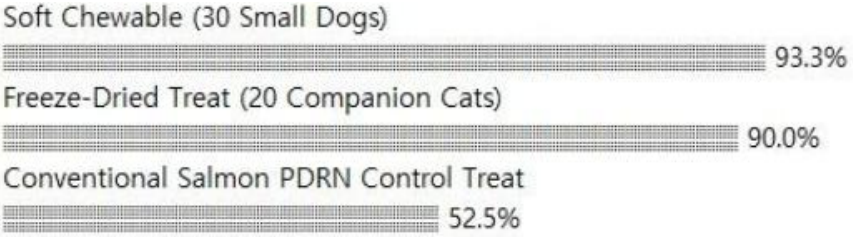

Figure 7: Palatability (feeding completion rate) evaluation results of Phyto-PDRN functional formulations for small dogs and cats

Unlike seafood-derived salmon PDRN formulations that cause fish odor (palatability 52.5%), plant-based Phyto-PDRN-based soft chewables (30 small dogs) recorded a high feeding completion rate of 93.3%, and freeze-dried treats (20 cats) recorded 90.0%, demonstrating high palatability without fish allergy or aversion. The hardness of the soft chewable was precisely controlled to 12.5 ± 1.2 N, and the freeze-dried treat to 6.8 ± 0.5 N, enabling consumption without tooth damage (Figure 7).

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IV. Conclusion And Recommendaruons

This study demonstrated that highly purified plant-derived PDRN (Phyto-PDRN) can overcome the limitations of existing aquatic animal PDRNs and be utilized as a core active ingredient in various bio-applications.

• Cell-Cultured Meat Industry: Aloe vera PDRN directly binds to MyoD and Pax7 proteins in Hoengseong Hanwoo muscle satellite cells, maintaining 100% proliferation performance even in an environment where Fetal Bovine Serum (FBS) – which accounts for most of the cultured meat production cost – is reduced by 50%.

• Skin Cosmeceuticals: The Phyto-PDRN and mineral complex alleviated skin cytotoxicity from blue light hazards and significantly induced the expression of SOD1, filaggrin, loricrin, and collagen genes, maximizing skin barrier recovery and moisturizing functions.

• Veterinary Medicine and Pet Healthcare: Winter raspberry-derived Phyto-PDRN exhibited cartilage regeneration and NF-kB anti-inflammatory effects mediated by the adenosine A2A receptor, and defended against oxidative apoptosis in feline renal tubular cells. Furthermore, it succeeded in hypoallergenic, highly palatable formulation that completely excludes fish odor and allergies.

In conclusion, plant resource-based Phyto-PDRN purification technology and complex compositions are next-generation universal biomaterials that satisfy safety, economic feasibility, and ethics, and will exert high value across the clean food-tech, functional cosmetic, and veterinary healthcare industries.

This research paper was reconstructed by comprehensively compiling the empirical data from the patent application draft document "Cultured meat culture medium additive composition comprising Aloe vera PDRN as an active ingredient", empirical research results from the Asian Journal of Beauty and Cosmetology paper (Kim et al., 2023) supported by the Ministry of SMEs and Startups technology development support project (Project No.: S2594138), and the American Journal of Engineering Research (AJER) paper (Kim et al., 2026) conducted with support from the Ministry of SMEs and Startups (Grant No.: RS-2025-25440208) and the Ministry of Agriculture, Food and Rural Affairs.

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