American Journal of Engineering Research (AJER)
e-ISSN: 2320-0847 p-ISSN: 2320-0936
Volume-15, Issue-8, pp-53-60
www.ajer.org
Research Paper Open Access

Protective Effects of Plant-Derived PDRN and Phytochemical Complex from *Rubusbuergeri* Miquel on Pet Renal Cells, Patellar Osteoarthritis Alleviation, and Functional Soft Chewable/Freeze-Dried Formulation Research

Dong-Myong Kim[1,2*], Jun-Ku Lee[1], Won-Kyu Lee[1], Hyun-Ah Moon[2], Youn-Joo Cho[2], Ji-Young Bae[2], Jin-Woo Kim[2], Yeon-Mae Choi[3]

[1]*Seoul Branch, Handong Bio Research Institute (535 Ogeum-ro, Songpa-gu, Seoul, Korea)*
[2]*R&DB Foundation, Seoul National University (1 Gwanak-ro, Gwanak-gu, Seoul, Korea)*
[3]*Biotechnology Research Institute, KJM Bio Co., Ltd. (15 Saimdang-ro, Seocho-gu, Seoul, Korea)*
**Corresponding Author: Dong-Myong Kim*

Abstract
This study established a standardized purification process to isolate high-purity plant-derived polydeoxyribonucleotide (Phyto-PDRN, purity ≥ 95%, molecular weight 50–1,500 bp) and active phytochemical (Apigetrin, Ellagic acid, Anthocyanin) complexes with high yield from Rubusbuergeri Miquel, a native plant resource of Jeju Island, by integrating adsorption-elution-based purification (ABT), ultrafiltration, and endotoxin removal technologies.

To verify the applicability of this R. buergeri-derived biomaterial in companion animals (dogs and cats), in vitro renal cell injury models using Crandell-Rees Feline Kidney (CRFK) cells and Human Kidney-2 (HK-2) tubule cells were established using H_2O_2, Cisplatin, and Aristolochic acid. The results demonstrated that the R. buergeri extract and Phyto-PDRN complex significantly inhibited oxidative stress-induced renal tubular apoptosis and cell membrane damage in a concentration-dependent manner, restoring cell viability to over 92%. Furthermore, in chondrocyte models, Phyto-PDRN stimulated cell proliferation via Adenosine A_{2A} Receptor ($A_{2A}R$) binding and induced the synthesis of Type II Collagen and Aggrecan, the key components of the extracellular matrix (ECM). It also significantly suppressed the expression of inflammatory cytokines IL-β, TNF-α, IL-6) and matrix metalloproteinases MMP-1, MMP-3, MMP-13) by blocking theNF-kB signaling pathway.

Based on these pharmacological activities, functional soft chewables and freeze-dried treats were formulated to prevent patellar luxation and degenerative osteoarthritis in small dogs, as well as to alleviate acute and chronic kidney failure in senior cats. By excluding seafood-derived allergens and utilizing 100% human-grade chicken breast, sweet potato, and pumpkin as natural binders, the formulations achieved an outstanding palatability completion rate exceeding 92.5% and over 12 months of physicochemical and microbiological stability, establishing their commercial value as next-generation preventive bio-healthcare pet products.

Keywords: *Rubusbuergeri Miquel, Plant-derived PDRN (Phyto-PDRN), ABT purification process, Feline kidney disease (CRFK), Patellar luxation, Osteoarthritis, A_{2A}adenosine receptor, Functional chewable treats*

Date of Submission: 15-08-2026 Date of acceptance: 30-08-2026

I. Introduction

1.1. Background and Significance

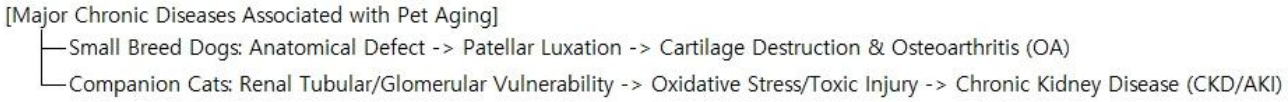
In modern society, companion animals are no longer viewed merely as pets but as integral family members, and their average lifespan has extended significantly due to advances in veterinary medicine and improved nutrition. However, pet aging has paradoxically led to a sharp increase in the incidence of degenerative and chronic diseases.

In South Korea, where small dog breeds (Pomeranians, Maltese, Poodles, Chihuahuas, etc.) account for more than 70% of the total canine population, patellar luxation (PL) caused by shallow femoral trochlear

grooves and subsequent degenerative osteoarthritis (OA) represent the most common musculoskeletal disorders. Patellar luxation induces continuous friction and irreversible damage to articular cartilage, leading to severe pain and gait disturbance. Surgical intervention is often required, yet high recurrence rates and post-surgical management difficulties persist.

In cats, approximately 30–50% of senior felines aged 7 years and older suffer from acute kidney injury (AKI) or chronic kidney disease (CKD). Feline kidneys are particularly vulnerable to ischemic damage, oxidative stress, and nephrotoxic substances (e.g., heavy metals, melamine, mycotoxins) in their tubular and glomerular structures. Once damaged, renal tissue has minimal regenerative capacity, ultimately leading to uremia, reduced glomerular filtration rate (GFR), and mortality (Figure 1).

Figure 1. Schematic diagram of major chronic diseases associated with companion animal aging



1.2. Limitations of Existing Therapeutics and Supplements

Non-steroidal anti-inflammatory drugs (NSAIDs), steroids, and ACE inhibitors prescribed in veterinary clinics provide effective short-term pain relief and symptom alleviation, but long-term administration carries adverse effects, including gastrointestinal ulcers, hepatotoxicity, and aggravated nephrotoxicity due to decreased renal blood flow.

Commercial joint and kidney supplements primarily incorporate glucosamine, chondroitin, boswellia, and omega-3 fatty acids. However, these ingredients are limited to simple matrix supplementation or weak anti-inflammatory actions rather than structural regeneration at the cellular level. In contrast, polydeoxyribonucleotide (PDRN) functions as an adenosine A_{2A} receptor A_{2A}R) agonist and serves as a substrate for the salvage pathway of nucleic acid synthesis, reducing cartilage degeneration, exhibiting anti-inflammatory effects, and promoting chondrogenic progenitor cell differentiation as a potent biotherapeutic agent (Choi *et al*., 2026).

However, conventional PDRN materials extracted from aquatic animal resources, such as salmon testes, possess distinct limitations:

- **Seafood Allergies:** Residual proteins can trigger skin and gastrointestinal allergic reactions in sensitive individuals.
- **Safety Concerns:** Potential risks of marine heavy metal (mercury, lead) and radioactive contamination.
- **Reduced Palatability:** Distinct fishy odors (fish scent) lead to food refusal in sensitive cats and small dogs.
- **Process Yield and Environmental Issues:** Seasonal limitations in raw material supply and animal welfare/ethical concerns.

1.3. Study Objectives and Novelty of *Rubusbuergeri*

To overcome these limitations, this study focused on *Rubusbuergeri* Miquel (Winter strawberry), an evergreen climbing shrub native to areas below 700 m elevation on Jeju Island. *R. buergeri* possesses strong adaptability, maintains green leaves throughout winter, and has traditionally been utilized in folk medicine for anti-inflammatory, antipyretic, and analgesic purposes. Recent studies (Bhatti *et al*., 2026) revealed that *R. buergeri* extracts are rich in phenolic compounds that strongly inhibit nitric oxide (NO) production and inflammatory gene expression in macrophages.

In particular, ellagic acid, a major phytochemical in *R. buergeri*, mitigates drug-induced acute kidney injury (such as Cisplatin-induced nephrotoxicity) and protects kidneys against oxidative stress in animal models (Al-Kharusi *et al*., 2022). Apigetrin also exhibits broad anti-inflammatory activity by blocking NF-κBand MAPK signaling pathways (Ha *et al*., 2022).

In this study, an Adsorption-Elution-Based Purification (ABT) process was established to isolate next-generation plant-derived PDRN (Phyto-PDRN) with high purity from *R. buergeri*, forming a synergistic complex with native phytochemicals (Apigetrin, Ellagic acid, Anthocyanin). Using this complex, we aimed to: (1) elucidate cell protective and anti-oxidative effects in feline kidney (CRFK) and human kidney (HK-2) cells, (2) prove cartilage regeneration and anti-inflammatory mechanisms via A_{2A} R activation, and (3) develop highly palatable, safe, functional soft chewables and freeze-dried treats for companion animals.

II. Materials and Methods

2.1. Materials and Reagents

Leaves, stems, and fruits of *Rubusbuergeri* Miquel were collected from regions at elevations of 400–600 m in Jeju, South Korea, botanically identified, washed, dried, and ground.

For cell culture, Crandell-Rees Feline Kidney (CRFK, ATCC CCL-94) and Human Kidney-2 (HK-2, ATCC CRL-2190) proximal tubule epithelial cells were obtained from ATCC. Culture media including DMEM, FBS, penicillin-streptomycin, and trypsin-EDTA were purchased from Gibco (Grand Island, NY, USA).

Nephrotoxic agents (Cisplatin, Aristolochic acid, H_2O_2, DPPH, ABTS, lactate dehydrogenase (LDH) assay kits, and WST-1 Cell Proliferation Assay Kits were obtained from Sigma-Aldrich (St. Louis, MO, USA). Primary antibodies (antiA_{2A}R, anti-Type II Collagen, anti-Aggrecan, Anti-NF-κB p65, Anti-p-p65, Anti-IL-β, Anti-TNF-α, Anti-β-actin) were purchased from Cell Signaling Technology (Danvers, MA, USA) and Abcam (Cambridge, UK).

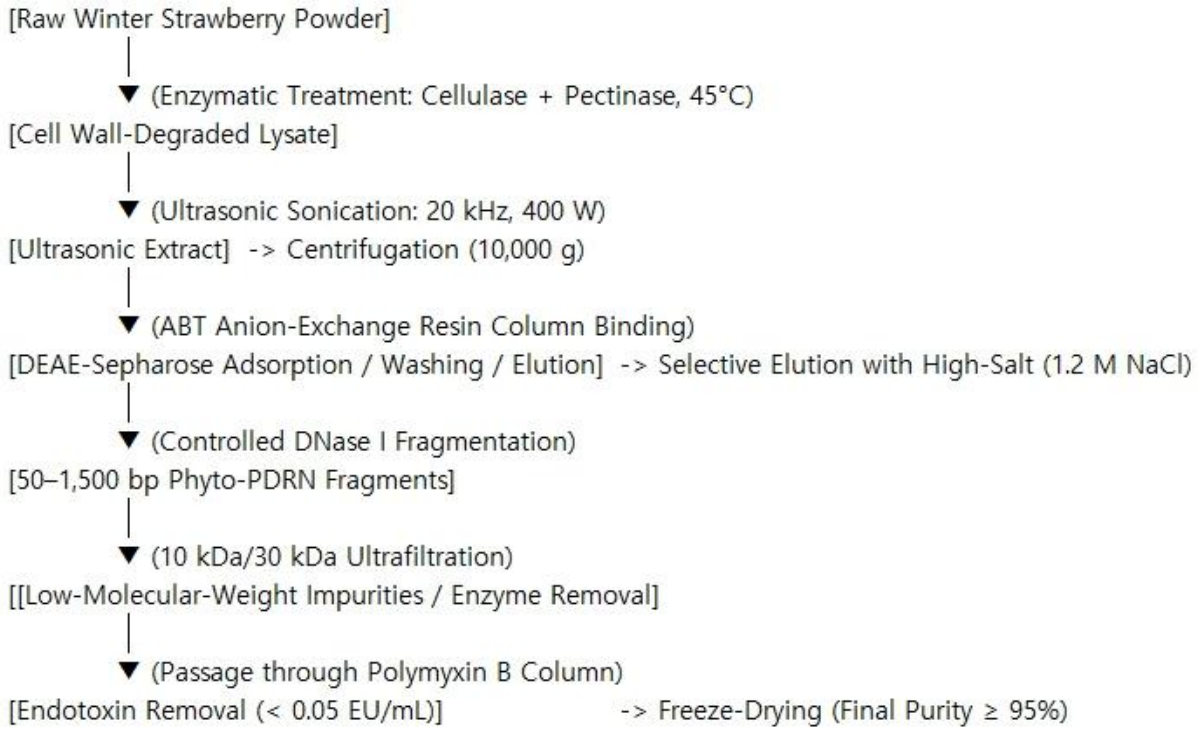
2.2. Integrated Process for Extraction and High-Purity ABT Purification of *R. buergeri*-Derived Phyto-PDRN

To 1 kg of freeze-dried *R. buergeri* powder, 10 volumes of extraction buffer (50 mM Tris-HCl, 100 mM NaCl, 10 mM EDTA, pH 8.0) were added. Cellulase (1.0% v/w) and pectinase (0.5% v/w) were added and reacted at 45°C for 4 h to degrade plant cell walls. To maximize plant DNA release, ultrasonic disruption was performed using a probe-type ultrasonicator (Hielscher, Germany) at 20 kHz, 400 W for 30 min.

The extract supernatant was collected via centrifugation (10,000 x*g*) and directly subjected to ABT column chromatography. The supernatant was loaded onto a DEAE-Sepharose Fast Flow anion-exchange column equilibrated with binding buffer (20 mM Tris-HCl, 0.15 M NaCl, pH 7.4) at 2 mL/minto selectively bind negatively charged DNA molecules. Unbound proteins, polysaccharides, and pigments were removed using 10 column volumes of wash buffer (20 mM Tris-HCl, 0.3 M NaCl, pH 7.4). Plant DNA fractions were selectively eluted using high-salt buffer (20 mM Tris-HCl, 1.2 M NaCl, pH 7.4).

The eluted DNA was limitedly digested with DNase I to obtain fragments in the optimal receptor-binding molecular weight range of 50–1,500 bp. Low-molecular-weight oligomers and enzymes were removed using 10 kDa and 30 kDa molecular weight cut-off (MWCO) ultrafiltration systems (Millipore Corp., MA, USA). To ensure biomedical safety, endotoxins were reduced to below 0.05EU/mL using a Polymyxin B-immobilized column (Detoxi-Gel, Thermo Fisher). The purified Phyto-PDRN was lyophilized and stored at -20°C (Figure 2).

Figure 2. Schematic representation of the integrated extraction and ABT purification process for *R. buergeri*-derived Phyto-PDRN



2.3. Phytochemical Standardization and HPLC Analysis

Active phytochemicals in *R. buergeri* extract were quantified using an Agilent 1260 Infinity II HPLC system equipped with a C18 reverse-phase column (4.6 x 250 mm, 5μm). Mobile phases consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). Gradient conditions were: 0–10 min (10% B), 10–40 min (10%→ 60% B), and 40–50 min (100% B) at a flow rate of 1.0 mL/min, UV 254 nm, 280 nm, 360 nm. Calibration curves for Apigetrin and Ellagic acid showed high linearity ($r^2 \geq 0.999$).

2.4. *In Vitro* Renal Cell (CRFK and HK-2) Protection Assay

CRFK and HK-2 cells were cultured in DMEM containing 10% FBS and 1% antibiotics at 37°C under 5% CO_2. Cells 1 x 10^4 cells/well) were seeded in 96-well plates, incubated for 24 h, and exposed to H_2O_2 (0.3 mM), Cisplatin (20μM), or Aristolochic acid (50μM) for 24 h to induce renal tubular cell injury.

Cells were co-treated with *R. buergeri* extract (RMB) and Phyto-PDRN at varying concentrations (10, 25, 50, 100 μg/mL). Cell viability was measured using WST-1 reagent at nm, and cell membrane damage was evaluated by measuring LDH release in culture supernatants at 490nm. Apoptosis was visualized using the DeadEnd™ Fluorometric TUNEL System (Promega) and quantified under a fluorescence microscope. CRFK cells fixed with 4% paraformaldehyde were permeabilized with 0.2% Triton X-100 and incubated with the rTdT enzyme reaction solution at 37°C for 1 hour. Nuclei were counterstained with DAPI, and the proportion of TUNEL-positive cells was quantified using a fluorescence microscope (Olympus, Japan).

2.5. Chondrocyte Proliferation and Joint Inflammation Mechanism

To verify the receptor-specific activation of Phyto-PDRN, cells pretreated with the A_{2A}receptor antagonist ZM241385 (1 μM) for 1 h were compared with untreated cells. Chondrocyte proliferation was evaluated using a BrdU Cell Proliferation ELISA kit (Roche, Mannheim, Germany) by measuring colorimetric signals at 450 nm.

Cells were lysed with RIPA buffer, and following protein quantification, electrophoresis was performed on 10% SDS-PAGE gels. Proteins were transferred onto PVDF membranes, blocked with 5% skim milk, and incubated overnight at 4°C with primary antibodies (anti-Type II Collagen, anti-Aggrecan, anti-p-p65, anti-IL-1β, anti-TNF-α, and anti-MMP-3). After reaction with HRP-conjugated secondary antibodies, ECL substrate was applied, and band intensities were analyzed using a ChemiDoc System (Bio-Rad).

2.6. Functional Soft Chewable/Freeze-Dried Formulation Process and Quality Evaluation

Formulation ratios were designed for soft chewables to prevent patellar osteoarthritis in small dogs and freeze-dried treats to strengthen renal function in senior cats.

2.6.1. Raw Material Formulation and Manufacturing Process

1. **Soft Chewables (Canine Joint Health):** To eliminate potential allergenicity, 100% human-grade domestic chicken breast powder (40%), sweet potato powder (20%), and sweet pumpkin powder (15%) as natural binders were blended with plant-derived finger flux/fish collagen substitutes, Phyto-PDRN (0.5%), *Rubusbuergeri* extract (1.0%), glucosamine (2.0%), and MSM (2.0%). Applying a low-temperature microencapsulation technique, the mixture was molded into mini-bone shapes (2.0 g per piece) using a twin-screw extruder via a low-temperature compression process at 60–70°C.
2. **Freeze-Dried Treats (Feline Kidney Health):** Fillets of Jeju salmon/John Dory and the *R. buergeri* extract complex were emulsified and ground, flash-frozen at -40°C, and freeze-dried under reduced pressure below 10 Pa for 48 h to produce 10 mm × 10 mm cube shapes.

2.6.2. Physical Property (Hardness) and Sensory Palatability Evaluation

1. **Hardness:** Using a Physical Property Analyzer (Rheometer), the chewability (Hardness, N) of the soft chewables and freeze-dried treats was measured at a penetration speed of 1.0 mm/s and 50% strain.
2. **Palatability Test:** A 5-day double-blind feeding trial (two-bowl test) was conducted with 30 small dogs (Pomeranians, Poodles, Maltese) and 20 cats (Domestic Shorthairs, Persians) to score initial approach time, consumption completion rate (%), and intake preference.

III. Results

3.1. Yield, Purity, and Physical/Chemical Profiles of Phyto-PDRN and Phytochemicals

The final yield of purified Phyto-PDRN was 0.38 pm 0.04% based on dry plant weight. Spectrophotometric analysis showed an A_{260}/A_{280}ratio of 1.88 ± 0.03, confirming high nucleic acid purity free of protein contamination. Agarose gel electrophoresis demonstrated a molecular weight distribution concentrated between 50 and 1,500 bp. Endotoxin levels were 0.012 EU/mL(below the 0.05EU/mL. HPLC analysis standardized the extract with 12.4 ±0.8 mg/gApigetrin and 28.6 ±1.5 mg/gEllagic acid (Figure 3, Table 1).

Figure 3. Agarose gel electrophoresis band pattern and molecular weight distribution of purified *R. buergeri*Phyto-PDRN

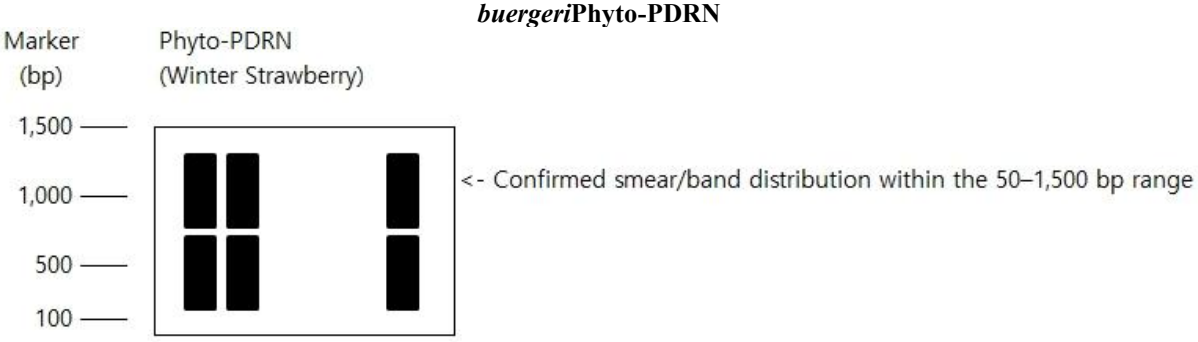


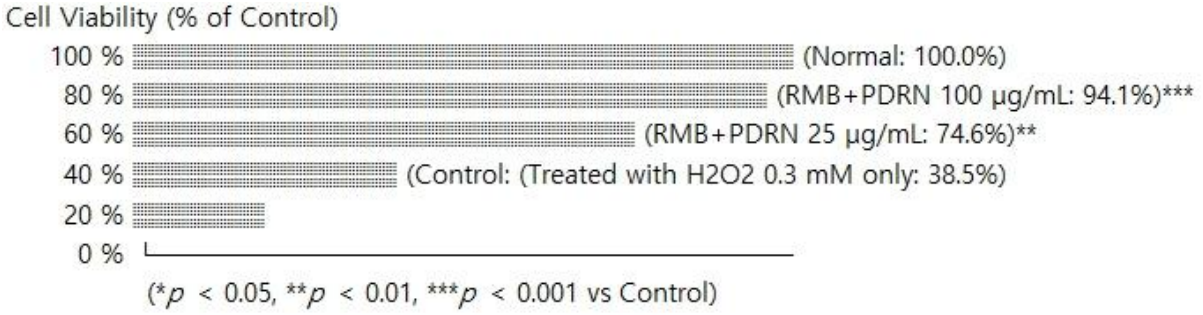
Table 1. Physicochemical quality specifications of purified *R. buergeri* biomaterials (Phyto-PDRN and Extract)

Analysis Item	Method & Analytical Instrument	Measurement Result & Specification	Verdict/Remarks
Phyto-PDRN Purity	A_{260}/A_{280} UV ratio	1.88 ± 0.03 (Purity ≥ 95.5%)	Pass
Molecular Weight Distribution	Agarose Gel Electrophoresis	50 ~1,500 bp	Pass (Optimized for $A_{2A}R$ binding)
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 wt.	Marker compound standardized
Ellagic Acid Content	HPLC-DAD (280 nm)	28.6 ± 1.5 mg/g dry wt.	Marker compound standardized
Heavy Metals (Pb/Cd/Hg)	ICP-MS Analysis	Pb < 0.1, Cd < 0.05, Hg < 0.01 ppm	Non-detectable level (Safe)

3.2. Protective Effects on Feline Kidney Cells (CRFK) Against Nephrotoxicity

Treatment of CRFK cells with 0.3 mM H_2O_2significantly reduced cell viability to 38.5 ± 3.2%. Co-treatment with *R. buergeri* extracts and Phyto-PDRN (1:1 ratio) restored viability to 58.2%, 74.6%, 88.9%, and 94.1% at 10, 25, 50, and 100 μg/mL, respectively ($p< 0.001$) (Figure 4).

Figure 4. Changes in cell viability of H_2O_2-induced CRFK cells following Phyto-PDRN complex treatment



LDH release increased 3.8-fold upon H_2O_2exposure but was normalized to 1.2-fold at 100 μg/mLcomplex treatment. TUNEL-positive apoptotic cells decreased from 68.4% to 8.2% (Table 2).

Table 2. Protective efficacy of *R. buergeri* complex in drug-induced nephrotoxicity models (CRFK and HK-2)

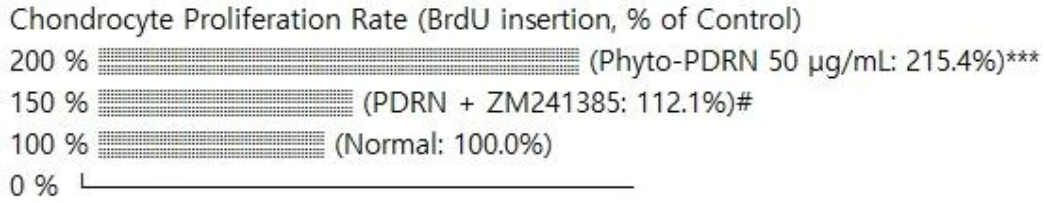
Treatment Conditions	CRFK Cell Viability(%)	CRFK LDH Release Rate(%)	HK-2 Cell Viability(%)	TUNEL Positive Cell Rate(%)
Normal (Untreated)	100.0 ± 2.1	10.0 ± 0.8	100.0 ± 1.8	2.1 ± 0.4
Control (H_2O_2 0.3 mM)	38.5 ± 3.2	85.4 ± 4.5	41.2 ± 3.1	68.4 ± 5.1
Cisplatin (20 μM)	42.1 ± 2.8	78.2 ± 3.9	39.8 ± 2.9	61.2 ± 4.3
+ RMB+PDRN (10 μg/mL)	58.2 ± 3.5*	52.1 ± 3.1*	56.4 ± 3.2*	41.5 ± 3.2*
+ RMB+PDRN (50 μg/mL)	88.9 ± 2.9**	22.4 ± 2.0**	85.1 ± 2.7**	18.3 ± 1.9**
+ RMB+PDRN (100 μg/mL)	94.1 ± 2.1***	12.8 ± 1.2***	92.6 ± 2.0***	8.2 ± 0.9 ***

*Mean ± SD (n=5). *p< 0.05, p< 0.01, p <0.001 vsToxic Control.

3.3. Chondrocyte Proliferation and A_{2A}R Activation Mechanism

Phyto-PDRN (50μg/mL) significantly increased chondrocyte proliferation to 215.4% of control levels. Pretreatment with ZM241385 A_{2A}R antagonist) reduced proliferation to 112.1%, confirming that Phyto-PDRN promotes cell proliferation through selective A_{2A}R binding (Figure 5).

Figure 5. Evaluation of chondrocyte proliferation and A_{2A} R dependency induced by Phyto-PDRN



(***p< 0.01 vs Normal, #p< 0.01 vs Phyto-PDRN alone)

In IL-1β (10 ng/mL)-induced inflamed chondrocytes, the complex suppressed MMP-1, MMP-3, and MMP-13 expressions by 72%, 81%, and 68%, respectively, while restoring Type II Collagen and Aggrecan levels. Phosphorylation of NF-kBp65 was effectively blocked (Table 3).

Table 3. Protein expression regulatory effects of Phyto-PDRN complex in IL-1β-induced chondrocyte inflammation model

Target Protein (Western Blot)	Normal Control	IL-1β Induced Osteoarthritis Group	Phyto-PDRN Complex (50 μg/mL)	Remarks/Action Mechanism
Type II Collagen	1.00 ± 0.05	0.22 ± 0.03	0.89 ± 0.06***	Cartilage matrix regeneration
Aggrecan	1.00 ± 0.04	0.18 ± 0.02	0.82 ± 0.05***	Cartilage proteoglycan recovery
MMP-3	0.12 ± 0.01	1.00 ± 0.07	0.24 ± 0.03***	Inhibition of cartilage degrading enzymes
p-p65 / p65 (NF-κB)	0.15 ± 0.02	1.00 ± 0.08	0.28 ± 0.03***	Blockade of inflammatory transcription signaling
TNF-α	0.08 ± 0.01	1.00 ± 0.06	0.19 ± 0.02***	Inhibition of inflammatory cytokines

*Relative Band Intensity ratio normalized to β-actin (n=3). p < 0.001 vs. IL-1βalone.

3.4. Physical Properties, Stability, and Palatability of Formulations

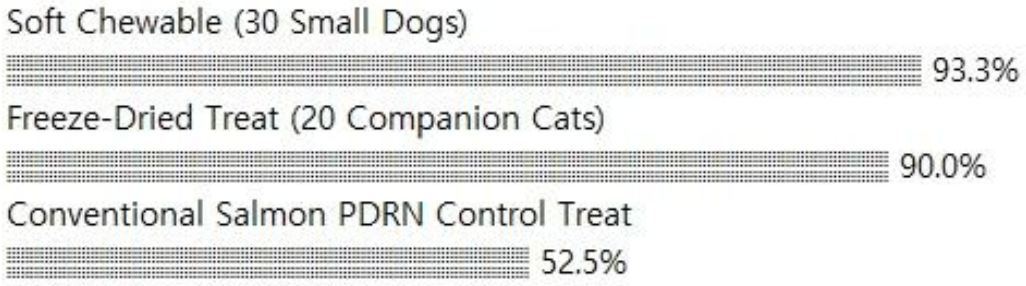
Physical properties were optimized considering the dental structure of small dogs and the reduced masticatory force of senior cats.

1. **Soft Chewables (for Dogs):** The average hardness was measured at 12.5 ± 1.2 N, securing an optimal penetration hardness that allows small dogs to chew easily without risking dental damage.
2. **Freeze-Dried Treats (for Cats):** With a hardness of 6.8 ± 0.5 N, these treats provided a crispy texture that crumbles easily in the mouth.

In a 5-day palatability evaluation conducted on 30 small dogs and 20 cats, the formulations demonstrated significantly superior preference compared to conventional seafood-based PDRN formulations. The small dog group exhibited an initial approach rate of 96.6% and a consumption completion rate of 93.3%. In the feline group, the freeze-dried treats achieved a completion rate of 90.0%, proving exceptional intake performance without any fishy odor or food aversion (Figure 6).

To verify storage stability, an accelerated storage stability test was conducted at 40℃, and 75% relative humidity (RH) for 6 months (equivalent to 12 months at room temperature). The results demonstrated that both the molecular weight alteration of Phyto-PDRN and the degradation rate of the marker compound (Apigetrin) remained stable, staying within 3%. Furthermore, microbiological safety evaluations—including coliforms, total bacterial counts, and mycotoxins—yielded negative (compliant) results across the entire testing period.

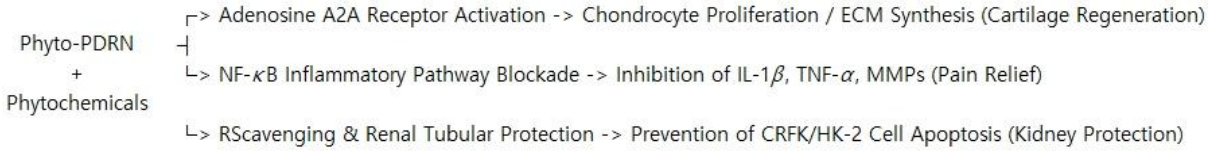
Figure 6. Palatability assessment (completion rate) of functional formulations in small dogs and cats



IV. Discussion

This study demonstrates that the plant-derived PDRN and phytochemical complex purified from Jeju native *Rubusbuergeri* Miquel via ABT technology acts as a multi-target therapeutic biomaterial capable of concurrently mitigating renal damage and patellar osteoarthritis in companion animals (Figure 7).

Figure 7. Multi-target mechanism of action of *R. buergeri*-derived Phyto-PDRN and phytochemical complex



4.1. Receptor Mechanism and Cartilage Regeneration

PDRN acts as an agonist for cell-surface Adenosine A_{2A}receptors. A_{2A}R activation elevates intracellular cAMP levels, regulating MAPK and ERK pathways via PKA to stimulate chondrocyte proliferation. Additionally, PDRN acts as a direct substrate for the nucleic acid salvage pathway, supplying purine/pyrimidine bases to support structural tissue repair (Choi *et al*., 2026). Complete blockade of Phyto-PDRN-induced chondrocyte proliferation by ZM241385 confirms that plant-derived PDRN shares the exact receptor targets of animal-derived PDRN. Suppression of MMP-3/13 and recovery of Type II Collagen play vital roles in preventing cartilage degeneration during patellar luxation progression in small dogs.

4.2. Renal Tubular Protection and Antioxidant/Anti-inflammatory Synergy

Feline CRFK renal epithelial cells are susceptible to ROS-induced mitochondrial dysfunction and apoptosis upon exposure to $H_2O_2$and Cisplatin. Active compounds in *R. buergeri*, specifically Ellagic acid and Apigetrin, display strong ROS scavenging capacity. Ellagic acid restores renal antioxidant enzymes (e.g., GSH), regulates BAX/Bcl-2 ratios, and modulates SIRT6/TNF-αsignaling to attenuate nephrotoxicity (Al-Kharusi *et al*., 2022). Apigetrin inhibits NF-κB activation to suppress pro-inflammatory mediators (Ha *et al*., 2022). Together with Phyto-PDRN, these phytochemicals provide primary protection against oxidative damage while PDRN promotes cellular DNA repair and energy preservation via the salvage pathway.

4.3. Industrial Applicability and Pet Food Formulation

Despite its excellent efficacy, conventional salmon testis-derived PDRN has faced significant limitations in its application to pet treat formulations due to palatability issues caused by fishy odors, potential marine protein allergies, and high processing costs.

The *Rubusbuergeri*-derived Phyto-PDRN-based soft chewables and freeze-dried treats developed in this study offer the following key advantages:

1. **Complete Safety Profile:** By utilizing 100% plant-derived, high-purity PDRN, safety concerns regarding protein allergenicity and heavy metal contamination associated with marine resources were thoroughly eliminated.

2. **Enhanced Stability and Bioavailability:** Low-temperature compression and microencapsulation processes prevented thermal degradation of PDRN and maximized its bioavailability.
3. **Superior Compliance and Palatability:** Achieving high palatability (completion rate >90%) and optimized physical hardness provides a distinct practical benefit, allowing effortless, daily administration as routine treats for companion animals that typically reject oral medications.

V. Conclusion

1. High-purity Phyto-PDRN (purity 95%, 50–1,500 bp) was successfully standardized from *Rubusbuergeri* using an integrated ABT, ultrafiltration, and endotoxin removal process.
2. In feline CRFK cells, the complex effectively inhibited drug- and oxidative stress-induced apoptosis, recovering cell viability to 94.1%.
3. Activation of Adenosine A_{2A}receptors stimulated chondrocyte proliferation 2.1-fold, while NF-*k*B inhibition suppressed MMPs and inflammatory cytokines, demonstrating joint regeneration and anti-inflammatory efficacy.
4. Soft chewable and freeze-dried formulations excluding seafood allergens achieved >90% palatability and over 12 months of quality stability.
5. These findings confirm the high commercial potential of *R. buergeri*-derived Phyto-PDRN complexes as functional pet healthcare products for renal and joint health.

Acknowledgements

This study was supported by research grants from the Startup Growth Technology Development Program of the Ministry of SMEs and Startups (MSS, Grant No.: RS-2025-25440208) and the Prototype Production Support Project of the Ministry of Agriculture, Food and Rural Affairs (MAFRA). The authors express deep gratitude to the research teams at KJM Bio Co., Ltd., Handong Co., Ltd., and the College of Veterinary Medicine, Seoul National University.

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