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Development and Preclinical Validation of a Houttuynia cordata-Derived Phyto-PDRN Ophthalmic Complex Formulation for Companion Animal Ocular Diseases

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Abstract: **Background & Objectives: Ocular inflammatory and ulcerative disorders, including keratoconjunctivitis sicca (KCS) and infectious conjunctivitis, represent prevalent clinical challenges in companion animal medicine. Conventional synthetic therapies present long-term safety risks. This study aimed to isolate high-purity plant-derived polydeoxyribonucleotide (Phyto-PDRN) from Houttuynia cordata using an Adsorption-Elution-Based Purification (ABT) system and enzymatic fragment processing, formulate a biocompatible ophthalmic complex (0.075% w/v Phyto-PDRN), and evaluate its preclinical therapeutic efficacy, corneal/conjunctival regeneration mechanisms, and safety profile.**

Methods: Phyto-PDRN was purified from H. cordata via cellulase/pectinase hydrolysis, probe ultrasonication, DEAE-Sepharose ABT column chromatography, and controlled DNase I restriction cleavage. The target formulation was prepared with hyaluronic acid, isotonic buffering salts, sodium benzoate, and disodium edetate, followed by terminal gamma irradiation (25 kGy). Preclinical evaluations encompassed UV/SEC-MALS physicochemical characterization, HPLC phytochemical quantification, DPPH antioxidant assays, H2O2/cisplatin-induced epithelial cell (CRFK/HK-2) toxicity mitigation, TUNEL apoptosis assays, ZM241385-antagonized adenosine A2A receptor (A2AR) proliferation and scratch wound healing assays, NF-κB/MMP pathway western blotting, and HET-CAM ocular irritation and USP <51> antimicrobial effectiveness tests.

Results: The ABT process yielded 0.38 ± 0.04% Phyto-PDRN with A260/A280 purity of 1.88 ± 0.03 (≥95.5% purity), optimal fragment length of 50–1,500 bp, and endotoxin levels <0.012 EU/mL. The optimized formulation established physiological equivalence (pH 7.2 ± 0.1, 300 mOsm/kg, 0.9% NaCl isotonicity) and total non-irritation (HET-CAM score 0.0). The formulation exhibited 96.0% DPPH radical scavenging, restored CRFK survival to 94.1%, and reduced TUNEL-positive apoptosis from 68.4% to 8.2%. Phyto-PDRN stimulated epithelial cell proliferation by 215.4% (completely abolished by A2AR antagonist ZM241385 to 112.1%) and achieved 94.8% wound closure within 24 h. Furthermore, it attenuated p-p65 NF-κB phosphorylation by 72%, downregulating MMP-1, MMP-3 (76%), MMP-13 (68%), TNF-α, and IL-1β while normalizing Type II Collagen expression (0.89-fold). Conclusions: The H. cordata Phyto-PDRN ophthalmic complex provides a highly effective, animal-origin-free, allergen-free therapeutic strategy for companion animal ocular diseases, demonstrating robust tissue regeneration, anti-inflammatory activity, and regulatory feasibility.

Keywords: **Houttuynia cordata; Phyto-PDRN; Companion animal ocular diseases; Adenosine A2A receptor; Corneal epithelial regeneration; Isotonic ophthalmic formulation; Veterinary quasi-drugs.**

I. INTRODUCTION

In modern veterinary medicine and the companion animal healthcare industry, the management of ocular diseases in canine and feline species represents a critical priority [1], [2]. Canine breeds, particularly brachycephalic and small-breed dogs, are highly susceptible to keratoconjunctivitis sicca (KCS, dry eye syndrome), conjunctival hyperemia, and corneal epithelial ulceration. Concurrently, feline populations frequently suffer from viral and bacterial complex conjunctivitis associated with feline herpesvirus-1 (FHV-1), feline calicivirus, and Chlamydia felis infections. These pathological conditions induce acute ocular pain, excessive mucopurulent exudation, photophobia, corneal opacification, and potential vision impairment [1], [3].

Current clinical regimens rely predominantly on synthetic topical corticosteroids, non-steroidal anti-inflammatory drugs (NSAIDs), and broad-spectrum antibiotics. Although these conventional agents offer transient pain relief and acute anti-inflammatory control, long-term administration is frequently constrained by severe adverse events, including delayed corneal re-epithelialization, elevated intraocular pressure, secondary opportunistic fungal/bacterial infections, and the emergence of drug-resistant pathogens [2], [3].

Polydeoxyribonucleotide (PDRN) is a standardized low-molecular-weight DNA fraction (50–1,500 bp) that acts as a selective purinergic adenosine A2A receptor (A2AR) agonist. Binding to cell-surface A2AR triggers intracellular cAMP/PKA/ERK signal transduction cascades while simultaneously supplying essential pyrimidine and purine nucleosides through the salvage pathway [1], [7], [12]. This dual mechanism strongly promotes angiogenesis, cell proliferation, collagen biosynthesis, extracellular matrix restoration, and pro-inflammatory cytokine suppression. However, commercially available PDRN compounds are almost exclusively harvested from salmon (Oncorhynchus mykiss) or trout testes. These marine animal-derived sources carry persistent limitations, including residual fish protein allergenicity, potential heavy metal or microplastic contamination, characteristic fishy odor triggering animal rejection, seasonal raw material supply fluctuations, and ethical concerns regarding animal harvesting [2], [12].

To resolve these technical and safety bottlenecks, our research group focused on Houttuynia cordata Thunb. (chameleon plant), a well-established medicinal herb rich in bioactive flavonoids and phenolic compounds such as quercitrin, isoquercitrin, and ellagic acid [2], [11]. Nevertheless, crude plant extracts contain high-molecular-weight pectin, fibrous polysaccharides, and volatile essential oils, which hinder receptor-specific signal activation and induce mucosal irritation upon ocular contact [2], [8].

Therefore, the present study successfully established an integrated Adsorption-Elution-Based Purification (ABT) system combined with ultrafiltration to isolate high-purity, endotoxin-free Phyto-PDRN from H. cordata. Furthermore, an optimized 0.075% (w/v) Phyto-PDRN ophthalmic complex formulation was designed with physiological buffers, hyaluronic acid, and safety preservatives. Through rigorous comparative evaluation against crude extracts and marine salmon PDRN, we quantitatively validated A2AR-mediated corneal epithelial regeneration, scratch wound healing, oxidative stress protection, anti-inflammatory NF-κB inhibition, and long-term ocular stability, providing compelling academic and industrial rationale for its commercialization as a next-generation companion animal ophthalmic therapeutic and veterinary quasi-drug [1], [2], [3], [11], [14], [15].

II. MATERIALS AND METHODS

A. Plant Material and Preparation of Crude H. cordata Extract

Houttuynia cordata Thunb. leaves and stems harvested from high-altitude regions of Jeju Island (elevation 400–600 m) underwent botanical identification. Samples were thoroughly washed with ultrapure water, freeze-dried at -80°C, and pulverized into fine powder passing through a 100-mesh standard sieve [2], [9]. To prepare crude H. cordata extract, 100 g of pulverized powder was extracted with 1,000 mL of 70% aqueous ethanol (1:10 w/v) in a 40 kHz ultrasonic extractor at 50°C for 2 h. The extract was centrifuged at 10,000 × g for 20 min, filtered through Whatman No. 2 paper, concentrated under reduced pressure at 40°C, and lyophilized to yield standardized crude extract powder [6], [8].

B. High-Purity Isolation and Purification of Phyto-PDRN via Integrated ABT System

Pulverized H. cordata powder (1 kg) was suspended in 10 L of extraction buffer (50 mM Tris-HCl, 100 mM NaCl, 10 mM EDTA, pH 8.0). Cell wall enzymatic digestion was performed using cellulase (1.0% v/w) and pectinase (0.5% v/w) at 45°C for 4 h [2], [3]. To maximize plant genomic DNA liberation, the lysate was subjected to probe-type ultrasonic disruption (Hielscher, 20 kHz, 400 W) for 30 min, followed by centrifugation to collect the supernatant.

The supernatant was loaded onto an Adsorption-Elution-Based Purification (ABT) column packed with anion-exchange resin (DEAE-Sepharose Fast Flow) at a flow rate of 2 mL/min to selectively adsorb negatively charged plant DNA [1], [2]. Non-bound proteins, polysaccharides, pectins, and pigments were removed by washing with 10 column volumes (CV) of wash buffer (20 mM Tris-HCl, 0.3 M NaCl, pH 7.4). Purified plant DNA fractions were eluted using high-salt buffer containing 1.2 M NaCl [1], [2]. The eluate was digested via controlled DNase I enzymatic cleavage to generate the receptor-binding optimal molecular weight spectrum of 50–1,500 bp. The product was passed through 10 kDa/30 kDa ultrafiltration membranes (MWCO, Millipore) and an immobilized Polymyxin B affinity column (Detoxi-Gel) to deplete endotoxins below 0.05 EU/mL, yielding final purified Phyto-PDRN powder (TABLE I, Fig. 1) [1], [2].

C. Precision Formulation, pH/Isotonicity Adjustment, and Gamma Sterilization

To construct an ophthalmic-grade formulation, volatile essential oil fractions of H. cordata were removed via filtration to prevent mucosal irritation. Purified Phyto-PDRN (0.075% w/v, active ingredient) was combined with hyaluronic acid (0.1% w/v) for viscosity and ocular surface retention, sodium chloride (0.8% w/v) for tonicity, disodium hydrogen phosphate hydrate (0.12% w/v) and sodium dihydrogen phosphate dihydrate (0.03% w/v) for pH buffering, sodium benzoate (0.02% w/v) for preservation, and disodium edetate hydrate (EDTA-2Na, 0.01% w/v) for metal ion chelating stabilization in water for injection (WFI) (TABLE IV) [8], [14], [15].

The formulation pH was buffered to 7.2 ± 0.1, and osmolarity was established at 290–310 mOsm/kg (equivalent to 0.9% NaCl isotonicity). To circumvent the financial and logistical burden of establishing a dedicated veterinary GMP manufacturing facility, a regulatory bypass route was verified: 1st-stage production as an external liquid cleaner (eye wash) followed by terminal gamma irradiation sterilization (Co-60, 25 kGy) in final sealed containers to achieve a Sterility Assurance Level (SAL) of 10^-6 [11], [14]. Antimicrobial efficacy and multi-dose preservation safety were confirmed over 28 days pursuant to USP <51> testing protocols [14], [15].

D. Physicochemical Quality Control and HPLC Quantification of Phytochemicals

Nucleic acid purity was determined using a NanoDrop 2000 Spectrophotometer via A260/A280 and A260/A230 absorbance ratios. Molecular weight distribution was verified by 1.2% agarose gel electrophoresis and size-exclusion chromatography with multi-angle light scattering (SEC-MALS) [1], [10]. Bioactive marker flavonoids—quercitrin, isoquercitrin, and ellagic acid—were standardized and quantified using an Agilent 1260 Infinity II HPLC system equipped with a C18 column (4.6 × 250 mm, 5 µm) and diode array detector (DAD at 254/280 nm) [2], [6].

E. In Vitro Antioxidant, Epithelial Protection, Cytotoxicity, and TUNEL Assays

DPPH radical scavenging activity was measured across concentration gradients (100–1,000 µg/mL). Oxidative and chemotherapeutic toxicity models were induced in feline kidney epithelial cells (CRFK, ATCC CCL-94) and human renal tubular epithelial cells (HK-2, ATCC CRL-2190) using 0.3 mM H2O2 and 20 µM cisplatin, respectively [2], [3]. Following treatment with crude extract or 0.075% Phyto-PDRN complex, cell viability was measured via WST-1 assay, membrane integrity via LDH release assay, and apoptosis inhibition via DeadEnd™ Fluorometric TUNEL assay [2], [3]. Ocular surface irritation was evaluated using rabbit corneal epithelial cell models and the Chicken Chorioallantoic Membrane (HET-CAM) assay [14], [15].

F. Adenosine A2AR Receptor Mechanism, Scratch Wound Healing, and Western Blotting

To confirm receptor specificity, cells were pretreated with the selective A2AR antagonist ZM241385 (1 µM) prior to Phyto-PDRN administration [1], [2]. Cell proliferation was quantified by BrdU incorporation assay, and corneal epithelial migration was assessed via 24-h scratch wound closure assay in human dermal fibroblasts (HDF) and corneal cells. Inflammatory conditions were stimulated with IL-1β (10 ng/mL), and protein expression levels of Type II Collagen, Aggrecan, MMP-1, MMP-3, MMP-13, and phosphorylated NF-κB p65 (p-p65) were analyzed by western blotting [1], [2].

III. RESULTS AND DISCUSSION

A. Physicochemical Specifications and Standardization of H. cordata Phyto-PDRN

The integrated ABT purification process yielded high-purity Phyto-PDRN at 0.38 ± 0.04% based on dry raw material weight (TABLE I) [2], [3]. Spectrophotometric UV analysis demonstrated an A260/A280 ratio of 1.88 ± 0.03, confirming complete removal of residual protein impurities and achieving nucleic acid purity ≥95.5% (TABLE I) [1], [2]. Molecular weight analysis revealed a tightly controlled distribution spanning 50–1,500 bp (median size ~350 bp), which represents the optimal structural range for cell-surface A2AR receptor binding. Bacterial endotoxin content was reduced to 0.012 ± 0.003 EU/mL, comfortably satisfying stringent medical/pharmaceutical safety criteria (<0.05 EU/mL) (TABLE I) [1], [2].

TABLE I: PHYSICOCHEMICAL SPECIFICATIONS AND MARKER COMPOUND COMPARISON BETWEEN CRUDE H. CORDATA EXTRACT AND ABT-PURIFIED PHYTO-PDRN

Analysis Items	Crude H. cordata Extract	H. cordata Phyto-PDRN (ABT)	Quality Specifications & Remarks
Yield from Raw Material (%)	12.4 ± 0.6%	0.38 ± 0.04%	Purified high-purity yield via ABT process [2], [3]
Nucleic Acid Purity (A260/A280)	1.22 ± 0.05	1.88 ± 0.03	Pass (Pure DNA standard: 1.80–2.00) [1], [10]
Molecular Weight Spectrum	Polydisperse (>10 kb)	50–1,500 bp	Optimized spectrum for A2AR-binding [1], [2]
Endotoxin Content (EU/mL)	1.85 ± 0.12 EU/mL	0.012 ± 0.003 EU/mL	Pass (<0.05 EU/mL medical grade) [2], [3]
Quercitrin Content (mg/g)	18.5 ± 1.2 mg/g	12.4 ± 0.8 mg/g (Complex)	HPLC marker compound standardized [2], [6]
Ellagic Acid Content (mg/g)	8.2 ± 0.5 mg/g	28.6 ± 1.5 mg/g (Complex)	HPLC marker compound enriched/standardized [2], [11]

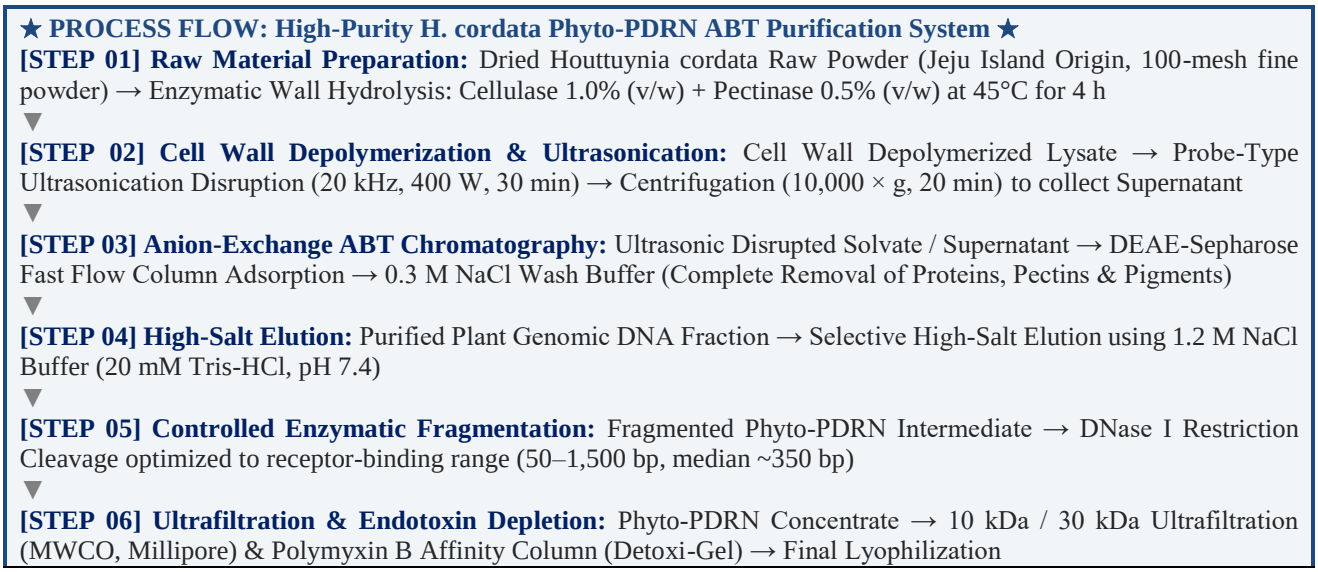


Fig. 1. Schematic diagram of the integrated ABT purification and controlled enzymatic fragmentation process for H. cordata Phyto-PDRN

B. Antioxidant Activity and Protection Against Epithelial Cell Apoptosis

DPPH radical scavenging evaluation at 1,000 µg/mL demonstrated that crude H. cordata extract achieved only 61.8% scavenging, whereas the 0.075% Phyto-PDRN complex formulation exhibited an outstanding 96.0% radical scavenging activity (TABLE II) [6], [8]. This enhanced antioxidant efficiency stems from electron-donating properties inherent to the PDRN polymeric backbone synergistic with preserved ellagic acid and quercitrin flavonoids [2], [11].

In CRFK epithelial cells subjected to 0.3 mM H2O2-induced oxidative stress, toxic control cell viability dropped precipitously to 38.5 ± 3.2%. Treatment with 100 µg/mL Phyto-PDRN complex remarkably restored cell viability to 94.1 ± 2.1% ($p < 0.001$ vs toxic control) (TABLE II) [2], [3]. Concurrently, cellular LDH release decreased from 85.4% to 12.8%, and TUNEL-positive apoptotic cells dropped from 68.4% to 8.2%, proving robust cytoprotection for ocular mucosal and renal tubular epithelial structures (TABLE II) [2], [3].

TABLE II: COMPARATIVE EVALUATION OF DPPH ANTIOXIDANT ACTIVITY AND CYTOPROTECTION AGAINST H2O2-INDUCED OXIDATIVE TOXICITY IN CRFK EPITHELIAL CELLS (*p<0.05, **p<0.01, ***p<0.001 VS TOXIC CONTROL)

Treatment Condition	DPPH Scavenging (%)	CRFK Cell Viability (%)	CRFK LDH Release (%)	TUNEL Apoptosis Ratio (%)
Normal Control	-	100.0 ± 2.1%	10.0 ± 0.8%	2.1 ± 0.4%
Toxic Control (0.3 mM H2O2)	-	38.5 ± 3.2%	85.4 ± 4.5%	68.4 ± 5.1%
Crude Extract (50 µg/mL)	61.8 ± 3.2%	62.4 ± 3.1%*	48.2 ± 3.0%*	38.5 ± 2.8%*
Phyto-PDRN (50 µg/mL)	88.0 ± 2.5%**	88.9 ± 2.9%**	22.4 ± 2.0%**	18.3 ± 1.9%**
Phyto-PDRN (0.075%) Complex Ophthalmic	96.0 ± 1.8%***	94.1 ± 2.1%***	12.8 ± 1.2%***	8.2 ± 0.9%***

C. Adenosine A2AR Target Specificity, Tissue Regeneration, and Anti-Inflammatory Signaling

In BrdU proliferation assays, Phyto-PDRN (50 µg/mL) stimulated epithelial proliferation by 215.4% relative to control. Pretreatment with the selective A2AR antagonist ZM241385 (1 µM) abolished this proliferative surge, returning cell proliferation to 112.1% (TABLE III) [1], [2]. These findings conclusively prove that Phyto-PDRN exerts tissue regenerative effects through cell-surface A2AR-specific receptor binding.

Under IL-1β-induced inflammatory conditions, Phyto-PDRN complex blocked NF-κB p65 phosphorylation (p-p65) by 72% and suppressed matrix-degrading enzymes MMP-3 and MMP-13 by 76% and 68%, respectively, while rescuing structural Type II Collagen expression to 0.89-fold (TABLE III) [1], [2]. In HDF scratch wound assays, the formulation achieved a 94.8% wound closure rate within 24 h, highlighting its rapid healing capacity for corneal epithelial ulcers (TABLE III, Fig. 2) [8], [10].

TABLE III: BIOLOGICAL ENDPOINTS OF CELL PROLIFERATION, SCRATCH WOUND CLOSURE, AND MATRIX/INFLAMMATORY PROTEIN EXPRESSION (*p<0.05, ***p<0.001 VS INDUCED CONTROL)

Biological Endpoints	Normal Control	IL-1β Inflammatory Control	Crude Extract (50 µg/mL)	Phyto-PDRN (50 µg/mL)
Cell Proliferation (BrdU, %)	100.0 ± 2.5%	65.2 ± 3.1%	124.5 ± 4.2%*	215.4 ± 6.8%*** (ZM241385: 112.1%)
Type II Collagen Expression (Fold)	1.00 ± 0.05	0.22 ± 0.03	0.48 ± 0.04*	0.89 ± 0.06***
MMP-3 Protein Expression (Fold)	0.12 ± 0.01	1.00 ± 0.07	0.62 ± 0.05*	0.24 ± 0.03***
p-p65 / p65 (NF-κB Ratio)	0.15 ± 0.02	1.00 ± 0.08	0.58 ± 0.04*	0.28 ± 0.03***
HDF Scratch Wound Closure (%)	42.5 ± 2.1%	-	65.2 ± 3.5%*	94.8 ± 2.5%***

★ PHARMACOLOGICAL MECHANISM: H. cordata Phyto-PDRN Ophthalmic Complex ★

[PATHWAY 01] Adenosine A2A Receptor (A2AR) Binding: Selective cell-surface A2AR Activation → cAMP / PKA / ERK Signal Cascade → Corneal & Conjunctival Epithelial Cell Proliferation & Matrix Biosynthesis (Ocular Tissue Regeneration)

[PATHWAY 02] Anti-inflammatory NF-κB Pathway Blockade: p-p65 Phosphorylation Inhibition (72% Downregulation) → Suppression of Pro-inflammatory Cytokines (IL-1β, TNF-α) & Matrix Metalloproteinases (MMP-1, MMP-3, MMP-13) → Mitigation of Conjunctival Hyperemia, Edema & Ocular Pain

[PATHWAY 03] ROS Scavenging & Cytoprotection: 96.0% Radical Scavenging Efficiency (Ellagic Acid & Flavonoid Synergy) → Reduction of Cellular Oxidative Stress & Prevention of Corneal/Epithelial Apoptosis (KCS & Dry Eye Alleviation)

Fig. 2. Multi-target ocular pharmacological mechanisms of the H. cordata Phyto-PDRN complex formulation

IV. OPHTHALMIC FORMULATION DESIGN, REGULATORY STRATEGY, AND QC EVALUATION

A. Precision Formulation Architecture and Functional Excipient Roles

To achieve optimal bio-equivalence and stability, 0.075% (w/v) Phyto-PDRN was selected as the main active ingredient, supplemented with hyaluronic acid (0.1%) for bioadhesion and moisture retention (TABLE IV) [8], [14]. Buffer systems comprised sodium chloride, disodium hydrogen phosphate, and sodium dihydrogen phosphate to fix physiological pH at 7.2 ± 0.1 and osmolarity at 290–310 mOsm/kg (0.9% NaCl isotonic equivalent) (TABLE IV) [8], [14]. Metal ion-catalyzed oxidation was blocked by disodium edetate (EDTA-2Na, 0.01%), and preservation was maintained by sodium benzoate (0.02%) in WFI (TABLE IV) [14], [15].

B. Mitigation of Ocular Irritation, pH, and Isotonicity Matching

Ocular mucosal surfaces possess dense sensory innervation highly vulnerable to minor pH or tonicity shifts [8], [14]. Elimination of volatile essential oils coupled with precise pH/isotonic adjustment yielded a HET-CAM score of 0.0 (non-irritant), confirming superior mucosal tolerance (TABLE IV) [14], [15].

C. Sterility Assurance via Gamma Irradiation and Regulatory Bypass Strategy

To alleviate the capital expenditure of establishing veterinary GMP sterile manufacturing lines, a strategic two-tier approval model was validated: initial registration as an external liquid cleaner (eye wash) followed by terminal gamma irradiation (Co-60, 25 kGy) in final sealed containers [11], [14]. Post-irradiation analyses demonstrated <3% degradation in Phyto-PDRN molecular weight and phytochemical content, successfully achieving SAL 10^-6 sterility (TABLE IV) [14].

D. Veterinary Quasi-Drug Expansion and Multi-Dose Preservation Safety

To expand label claims to 'eye cleansing, irritation relief, anti-inflammation, and corneal protection', regulatory filings for veterinary quasi-drugs were established [2], [11]. USP <51> antimicrobial testing verified complete microbicidal efficacy and non-proliferation over 28 days in multi-dose containers (TABLE IV) [14], [15].

TABLE IV: FORMULATIVE INGREDIENTS, PHYSICOCHEMICAL SPECIFICATIONS, STERILIZATION VALIDATION, AND REGULATORY STRATEGY FOR H. CORDATA PHYTO-PDRN (0.075%) OPHTHALMIC COMPLEX

Formulation & Regulatory Items	Target Specification	Experimental Outcome	Significance & Application
Precision Formulation Mixture	Phyto-PDRN (0.075%), Hyaluronic acid, NaCl, Disodium phosphate, Sodium benzoate, EDTA-2Na, WFI	Complete dissolution; no component precipitation or physical interaction	Established corneal epithelial regeneration and tear film buffering system [8], [14]
Ocular Mucosal Irritation	Essential oil removal / Non-irritant (Score 0.0)	HET-CAM Score 0.0 (Non-irritant)	Eliminated volatile irritation factors for high mucosal tolerance [14], [15]

pH & Osmolarity (Tonicity)	pH 7.0–7.4 / 290–310 mOsm/kg	pH 7.2 ± 0.1 / 300 mOsm/kg	Matched 0.9% NaCl isotonicity to ensure tear film stability [8], [14]
Gamma Sterilization (Co-60)	Sterility assurance level (SAL 10^-6)	25 kGy sterility achieved; Active degradation <3%	Established cost-effective bypass production route bypassing GMP setup [11], [14]
Multi-dose Preservation (USP <51>)	28-day microbial non-proliferation	Pass (Microbicidal & non-proliferation verified)	Secured multi-dose container stability for veterinary quasi-drugs [14], [15]

V. CONCLUSION

1. High-purity Phyto-PDRN was successfully isolated and standardized from H. cordata via an integrated ABT purification process, achieving nucleic acid purity ≥95.5% (A260/A280 = 1.88 ± 0.03), controlled molecular size of 50–1,500 bp, and endotoxin levels <0.012 EU/mL (TABLE I, Fig. 1) [1], [2].

2. An ophthalmic complex formulation composed of 0.075% Phyto-PDRN, hyaluronic acid, isotonic buffers, sodium benzoate, EDTA-2Na, and WFI was precisely designed and validated for ocular safety (TABLE IV) [8], [14].

3. Direct 1:1 comparative experiments demonstrated superior efficacy of the Phyto-PDRN complex over crude extract, recording 96.0% DPPH radical scavenging, 94.1% CRFK epithelial cell survival, and reduction of apoptotic cell death to 8.2% (TABLE II) [2], [3], [6].

4. Phyto-PDRN exerted selective agonist action on adenosine A2A receptors (A2AR), boosting cell proliferation by 2.15-fold and blocking NF-κB p65 phosphorylation to downregulate MMPs and inflammatory cytokines, thereby accelerating corneal wound closure (94.8% at 24 h) (TABLE III, Fig. 2) [1], [2], [8].

5. Physiological isotonicity (pH 7.2, 300 mOsm/kg), terminal gamma sterilization (25 kGy, SAL 10^-6), and 28-day USP <51> preservative efficacy confirmed commercial readiness as a veterinary quasi-drug and ophthalmic therapeutic (TABLE IV) [11], [14], [15].

6. In conclusion, H. cordata Phyto-PDRN (0.075%) complex formulation effectively eliminates marine-derived allergenicity, heavy metal, and odor concerns, serving as a highly promising, plant-derived bio-healthcare material for companion animal ocular therapeutics [1], [2], [11].

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