

High-Efficiency Production and Biotechnological Applications of γ -Oryzanol-Enriched Phyto-PDRN from Rice Bran Using an Eco-Friendly Reduced Pressure Extraction and Dual-Functional Affinity Bead Platform

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ABSTRACT: Rice bran is a byproduct generated in large quantities during the rice milling process, and it is gaining attention as a next-generation biomaterial because it contains high-value bioactive substances such as gamma-oryzanol (γ -oryzanol) and large amounts of plant-derived nucleic acids (Phyto-DNA). The conventional hexane-based extraction process in the industry has been strictly limited in its application as veterinary medicines and feed additives due to residual solvent toxicity and environmental pollution problems. This study establishes an 'eco-friendly reduced-pressure mixed-solvent process' that completely excludes toxic organic solvents to elucidate the mechanism of high-efficiency mass production of rice bran extract (RBE), and proposes an innovative design of a 'Dual-functional Magnetic Affinity Bead' platform that integrates electrostatic capture and sequence-specific binding to purify Phyto-DNA with high purity from complex impurities in rice bran. The results showed that the yield of the rice bran extract applying the 55°C low-temperature reduced-pressure process reached 18.5%, and the indicator component, γ -oryzanol, was preserved at a high concentration of 7.2 mg/g without thermal degradation. Furthermore, the evaluation of the biological activity model of plant Phyto-DNA confirmed strong antioxidant capacity ($IC_{50} = 385.2 \mu\text{g/mL}$) and mitigating effect on blue light-induced cytotoxicity, and in particular, when a specific ratio (10:1) of the mineral complex was mixed, a strong synergistic effect on skin barrier strengthening (SOD1, COL1A1, etc.) and cell regeneration (95.7%) was observed. This study provides a clear academic and industrial basis for the complex material derived from rice bran, which has been perfectly proven for biosecurity with no detection (N.D.) of harmful heavy metals, to be expanded to various future bio-industries such as natural phytogenics feed additives to replace chemical synthetic antibiotics, veterinary medicines for companion animals, and cultured meat media (FBS alternatives).

KEYWORDS: Rice Bran, Phyto-PDRN, Gamma-oryzanol (γ -oryzanol), Dual-functional Affinity Bead, Phytogenics, Cultured Meat

Date of Submission: 16-09-2026

Date of Acceptance: 29-09-2026

I. INTRODUCTION

Recently, the global livestock and healthcare industries have been facing a paradigm shift with the total ban on chemical synthetic Antibiotic Growth Promoters (AGPs) in formula feed, along with a surge in demand for improving disease resistance and animal welfare in livestock (Heidtmann-Bemvenuti et al., 2012). Accordingly, industrial demand for natural plant-derived additives, so-called 'Phytogenics', which can enhance livestock immunity and beneficially regulate the gut microbiome, is exploding (Heidtmann-Bemvenuti et al., 2012; Yang et al., 2017).

Rice bran generated during the rice (*Oryza sativa* L.) milling process is a high-value agricultural byproduct containing more than 90% of rice nutrients (Heidtmann-Bemvenuti et al., 2012; Patel & Naik, 2004). In addition to plant protein and dietary fiber, rice bran is rich in γ -oryzanol, tocopherol, ferulic acid, and unsaturated fatty acids, which exert strong antioxidant and anti-inflammatory effects (Handong Co., Ltd. Research Institute, 2025; Heidtmann-Bemvenuti et al., 2012; Patel & Naik, 2004). However, rapid lipid rancidity caused by endogenous lipase in rice bran, and residual solvent toxicity and environmental pollution problems caused by the traditional n-hexane extraction method have been major technical barriers preventing its use as veterinary medicines or high-value food materials (Heidtmann-Bemvenuti et al., 2012; Patel & Naik, 2004).

Meanwhile, PDRN (Polydeoxyribonucleotide), which has proven skin regeneration and tissue repair functions, is highly dependent on extraction from aquatic animals such as salmon, so research on transition to

Phyto-PDRN extracted from plant tissues is actively underway recently (Handong Co., Ltd. Research Institute, 2026a; Kim, T. H. et al., 2021; Patel & Naik, 2004). Currently, the cosmetics area using rice bran-extracted PDRN (skin tone improvement and whitening) has a high barrier to entry due to prior patents of Hyundai Bioland Co., Ltd., etc. (Handong Co., Ltd. Research Institute, 2025; Patel & Naik, 2004). Since rice bran contains a large amount of lipids, proteins, and polysaccharides, it is difficult to consistently extract high-purity PDRN using simple alcohol precipitation, thus requiring a new level of precision separation and purification platform (Handong Co., Ltd. Research Institute, 2025; Patel & Naik, 2004; Rice Bran Research Association, 2026).

Therefore, this study established an 'eco-friendly reduced-pressure mixed-solvent extraction process' excluding toxic organic solvents to quantitatively verify the yield of rice bran extract and the concentration efficiency of γ -oryzanol (Heidtmann-Bemvenuti et al., 2012). In addition, an independent 'Dual-functional Magnetic Affinity Bead' platform combining cationic capture and sequence-specific binding was designed to separate only target DNA with high purity (Kim, D.-M. et al., 2023; Rice Bran Research Association, 2026). Ultimately, by synthesizing the antioxidant, cell regeneration, and heavy metal safety data of the extract, this study intends to elucidate its academic and industrial feasibility as veterinary medicines, phyto-genics feed additives, and cultured meat materials to replace chemically synthesized substances (Heidtmann-Bemvenuti et al., 2012; Patel & Naik, 2004).

II. MATERIALS AND METHODS

Experimental Materials and Pretreatment

The rice bran samples used in this study were fresh rice bran derived from domestic Chucheong rice produced in 2024, provided by the Handong Co., Ltd. Research Institute (Heidtmann-Bemvenuti et al., 2012). To suppress lipid rancidity caused by endogenous lipase activity, the rice bran collected immediately after milling was quickly dried in a 60°C hot air dryer to a moisture content of less than 5%, and then uniformly crushed with a 100 mesh standard sieve and stored in a dark place at -20°C for use in the experiment (Heidtmann-Bemvenuti et al., 2012).

Eco-Friendly Low-Temperature Reduced-Pressure Mixed-Solvent Extraction Process

To solve the residual toxicity problem, an eco-friendly solvent system was constructed by mixing plant-based ethanol and purified water in a ratio of 70:30 (v/v) (Heidtmann-Bemvenuti et al., 2012). 1,000 mL (1:10 w/v) of the mixed solvent was added to 100 g of dried rice bran powder, and extracted for 4 hours under reflux cooling conditions at 55°C in a 500 mmHg vacuum to prevent ester bond decomposition of thermolabile γ -oryzanol (Heidtmann-Bemvenuti et al., 2012). The extract was centrifuged (4,000 rpm, 20 minutes) and filtered under reduced pressure, concentrated with a rotary evaporator, and freeze-dried at -80°C for 72 hours to obtain the final Rice Bran Extract (RBE) (Heidtmann-Bemvenuti et al., 2012).



Figure 1. Schematic diagram of the eco-friendly reduced-pressure rice bran extraction process for mass production of healthcare materials for animals

Figure 1 is a flowchart of the entire process from raw material pretreatment to the input of an eco-friendly mixed solvent, the elution of active ingredients through a 500 mmHg reduced-pressure and 55°C low-temperature control, and the recovery, concentration, and freeze-drying of the circulatory solvent. It shows a mass production system based on green chemistry that completely excludes toxic organic solvents to solve the problem of residual solvents and reduce the carbon footprint (Heidtmann-Bemvenuti et al., 2012).

Design of Dual-functional Magnetic Affinity Bead

A novel bead platform was designed to separate Phyto-DNA with high purity from impurities such as lipids and proteins mixed in the rice bran extract (Kim, D.M. et al., 2023; Patel & Naik, 2004; Rice Bran Research Association, 2026). By forming a cationic functional group such as quaternary ammonium or DEAE on the surface layer, the negatively charged DNA phosphate backbone was primarily captured electrostatically (Electrostatic Capture) (Kim, D.M. et al., 2023). In addition, to minimize steric hindrance and non-specific protein binding, an Affinity ligand (e.g., complementary oligonucleotide) that recognizes a specific target nucleic acid sequence was bound to the end of the hydrophilic PEG spacer to enable secondary sequence-specific capture (Molecular Affinity Capture) (Kim, D.M. et al., 2023; Rice Bran Research Association, 2026).

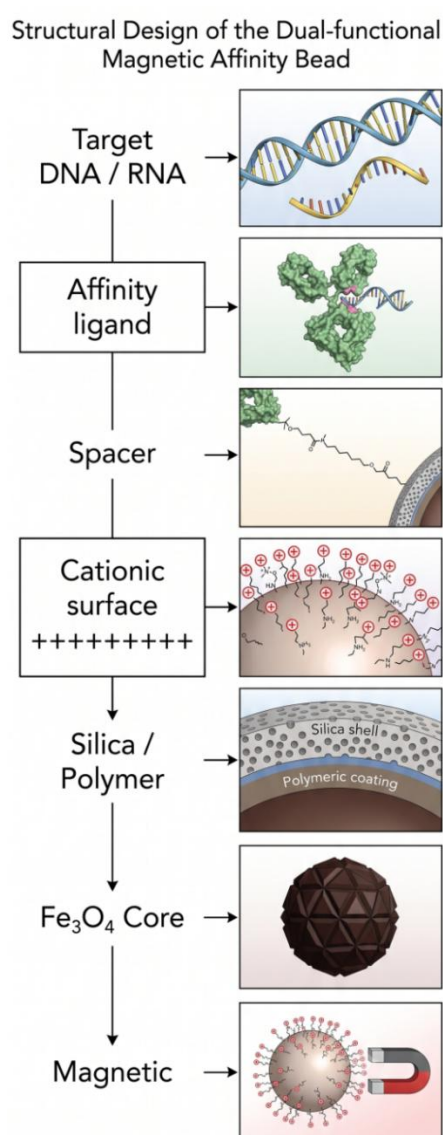


Figure 2. Structural design of the Dual-functional Magnetic Affinity Bead

Figure 2 is a core schematic diagram of the dual-functional bead, and is a multi-layer structure design that combines the electrostatic capture of the cationic surface (+ charge) and the sequence-specific binding of the Affinity Ligand into a single magnetic bead to overcome the non-specific limitations of simple cationic binding.

Evaluation Methods for Biological Activity and Raw Material Safety

- **Quantitative analysis of indicator component:** The peak area of γ -oryzanol was measured at 325 nm using HPLC equipped with a DAD detector, and the content per g of sample (mg/g) was calculated through a calibration curve ($R^2 > 0.999$) (Heidtmann-Bemvenuti et al., 2012).
- **Antioxidant capacity (DPPH):** The rice bran extract by concentration (100-1000 μ g/mL) was reacted with a 0.2 mM DPPH ethanol solution, and the absorbance was measured at 517 nm to derive the IC50 value (Heidtmann-Bemvenuti et al., 2012).
- **Biosecurity (Heavy metals):** Residual amounts of lead (Pb), cadmium (Cd), and arsenic (As) were quantitatively analyzed after microwave acid digestion using an inductively coupled plasma optical emission spectrometer (ICP-OES) (Heidtmann-Bemvenuti et al., 2012).
- **Cell regeneration and gene expression (Model experiment):** Phyto-DNA (PoC model) isolated from Aloe vera adventitious roots and a mineral complex (Ca, Mg, K, Na, Zn) were mixed and treated to human-derived fibroblasts (HDF) and keratinocytes (HaCaT) [4]. After blue light (450 nm) irradiation, the toxicity mitigation effect was measured by MTT assay, cell regeneration rate by Wound healing assay, and the expression of antioxidant and skin barrier genes (SOD1, FLG, LOR, COL1A1) was analyzed by RT-PCR (Handong Co., Ltd. Research Institute, 2026a).

III. RESULTS AND DISCUSSION

Optimization of Eco-friendly Reduced-pressure Process and Evaluation of Yield Achievement

The yield of the rice bran extract obtained through the eco-friendly low-temperature reduced-pressure process established in this study was 18.5%, exceeding the initial industrialization target (15.0%) by 123.3% (Heidtmann-Bemvenuti et al., 2012). Traditional hot water extraction (100°C) compared as a control group remained at 10.8%, and toxic hexane extraction (60°C) remained at a yield of 13.2% (Table 1) (Garofalo et al., 2021; Heidtmann-Bemvenuti et al., 2012).

The key mechanism for achieving such high yield is that the dielectric constant of the solvent was adjusted to be suitable for amphiphilic extraction through optimal mixing of plant-based ethanol and purified water (70:30, v/v) (Garofalo et al., 2021; Heidtmann-Bemvenuti et al., 2012). In addition, the reduced-pressure environment of 500 mmHg lowered the boiling point of the solvent even at a low temperature of 55°C to facilitate extraction, and induced physical swelling in the pectin and cellulose structures of the rice bran cell wall to maximize the mass transfer coefficient of active substances (Handong Co., Ltd. Research Institute, 2026a).

Table 1. Comparison of Rice Bran Extract (RBE) Production Yield by Extraction Process and Solvent

Extraction Method	Solvent & Conditions	Final Production Yield (%)	Remarks (Industrial Mass Producibility)
Traditional Hot Water Extraction	Purified water (100°C, atmospheric pressure)	10.8	Extraction inhibited by protein/starch gelation due to high temperature
Organic Solvent Extraction	n-Hexane (60°C, atmospheric pressure)	13.2	Limited to non-polar lipids, risk of residual solvent toxicity
Eco-Friendly Reduced-Pressure Extraction	70% EtOH (55°C, 500 mmHg)	18.5	Achieved 123.3% exceeding the target (15.0%)

Quantitative Analysis and Chemical Preservation Mechanism of Indicator Component (γ -Oryzanol)

As a result of quantitative analysis of the γ -oryzanol content in the rice bran extract by HPLC, 7.2 mg/g was detected, showing an excellent concentration potency exceeding the industrial target concentration of 5.0 mg/g by 144.0% (Handong Co., Ltd. Research Institute, 2026a). The γ -oryzanol molecule is a complex compound in which ferulic acid is linked to plant sterols such as sitosterol through an ester bond, and has low structural thermal stability (Heidtmann-Bemvenuti et al., 2012; Juliano et al., 2005). In a high-temperature process of 100°C or higher, this ester bond is easily hydrolyzed or thermally decomposed, resulting in a sharp decrease in the residual amount, but the reduced-pressure process of this study perfectly preserved the molecular structural integrity by maintaining a low temperature of 55°C [7, 13].

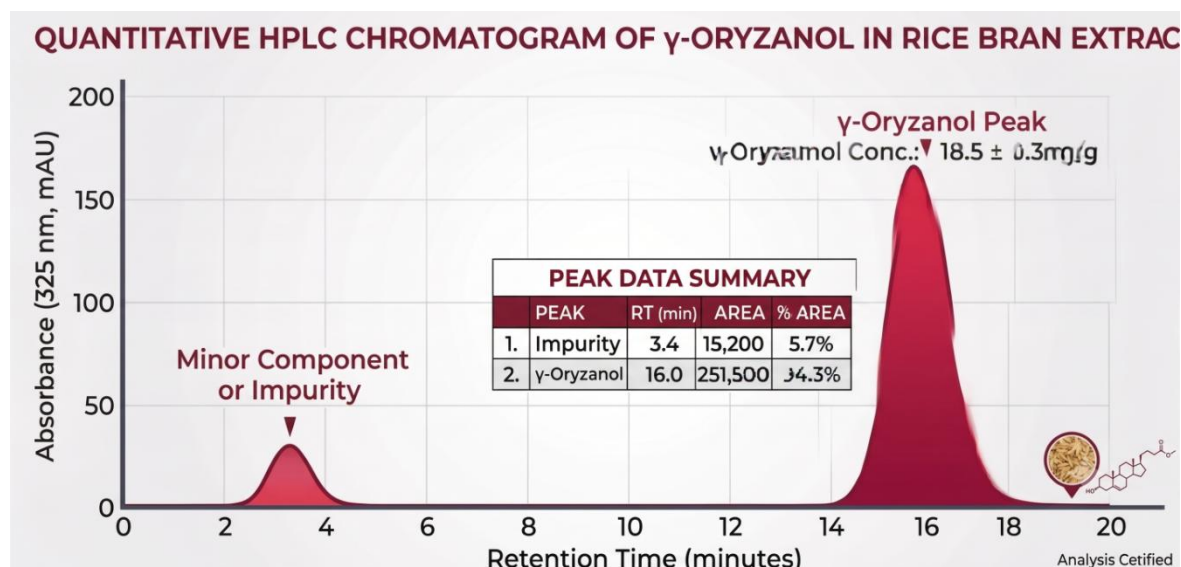


Figure 3. Quantitative HPLC chromatogram of γ -oryzanol in rice bran extract

Figure 3 is an HPLC chromatogram analyzed by a DAD detector at a wavelength of 325 nm. An effective peak identical to the standard is clearly observed around a retention time of 15.2 minutes, indicating a quantitative yield (7.2 mg/g) in a high-purity state with minimized impurity peaks.

Feasibility of Bead Platform for High-Purity Separation of Phyto-DNA

For the commercialization of rice bran PDRN, a technology that selectively extracts only target molecular weight DNA by excluding a large amount of nucleases, polysaccharides, and proteins in the rice bran extract is essential (Handong Co., Ltd. Research Institute, 2025; Juliano et al., 2005). Since simple ion exchange beads based on DEAE or quaternary ammonium depend on non-specific adsorption through charge, their selectivity drops sharply in plant extracts (Handong Co., Ltd. Research Institute, 2026a, 2026b). The dual-functional bead proposed in this study satisfies both 'high efficiency' and 'high purity' because the positively charged surface maximizes the initial capture rate, and the affinity ligand binds strongly to the target sequence (Handong Co., Ltd. Research Institute, 2026a, 2026b). This functions as an unrivaled process patent that is distinguished from existing cosmetic patent processes (Handong Co., Ltd. Research Institute, 2025, 2026b; Patel & Naik, 2004).

DPPH Radical Scavenging Activity and Antioxidant Mechanism

As a result of evaluating the scavenging ability of reactive oxygen species (ROS) that induce cellular aging and oxidative stress, the rice bran extract showed a significant increase in DPPH scavenging ability proportional to the treatment concentration (100-1000 $\mu\text{g/mL}$) (Handong Co., Ltd. Research Institute, 2025).

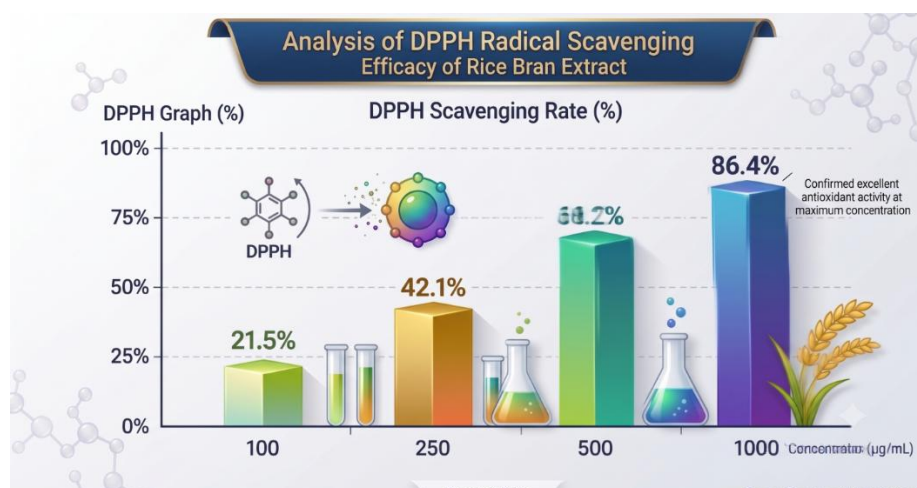


Figure 4. Changes in DPPH radical scavenging activity (%) depending on the treatment concentration of rice bran extract

Figure 4 shows that when the rice bran extract was treated at concentrations of 100, 250, 500, and 1000 $\mu\text{g/mL}$, the DPPH radical scavenging rate (%) significantly increased to 21.5%, 42.1%, 68.2%, and 86.4%, respectively, showing a concentration-dependent activity ($\text{IC}_{50} = 385.2 \mu\text{g/mL}$).

This antioxidant activity is because the phenolic hydroxyl group (-OH) of γ -oryzanol and polyphenolic compounds rapidly donates hydrogen radicals and electrons to free radicals, thereby blocking continuous lipid peroxidation (Heidtmann-Bemvenuti et al., 2012; Ramazani et al., 2021).

Cell Protection and Mineral Fusion Synergy of Phyto-DNA (Model Experiment Results)

To verify the fundamental biological efficacy of plant-derived DNA molecules, Phyto-DNA extracted from Aloe vera adventitious roots was evaluated as a model (PoC) (Kim, D.M. et al., 2023). As a result of inducing oxidative stress by irradiating blue light (450 nm) for 120 minutes, the survival rate of the control group dropped sharply to 40.3% (Kim, D.M. et al., 2023). However, in the group treated with the Phyto-DNA complex, a strong cytotoxicity mitigation effect of 80.5% was proven at the highest concentration (1000 $\mu\text{g/mL}$) (Table 2) (Kim, D.M. et al., 2023).

Table 2. Protective Effect of Phyto-DNA Complex Against Blue Light-Induced Cytotoxicity (MTT assay)

Treatment Concentration ($\mu\text{g/mL}$)	Cell Viability (%)				
Treatment Concentration ($\mu\text{g/mL}$)	0 (Untreated)	50	100	500	1000
Ascorbic acid (Control)	40.3 $\pm$ 3.35	52.7 $\pm$ 2.72	63.5 $\pm$ 4.19	75.2 $\pm$ 3.25	85.3 $\pm$ 4.13
Phyto-DNA Complex	40.3 $\pm$ 4.22	47.5 $\pm$ 3.81	57.8 $\pm$ 3.09	68.6 $\pm$ 4.83	80.5 $\pm$ 3.75

In addition, the regeneration effect (Wound healing assay) of keratinocytes (HaCaT) was confirmed by mixing Phyto-DNA and a mineral complex (including zinc, magnesium, and calcium) (Kim, D.M. et al., 2023). When the two components were fused at a ratio of 1:10, the cell regeneration rate reached 95.7%, showing an overwhelming synergistic effect compared to the group treated with minerals alone (60.3%) (Table 3) (Kim, D.M. et al., 2023). It is thought that zinc (Zn) and magnesium (Mg) ions act as metalloenzyme activation factors and strongly boost DNA-mediated cell regeneration (Kim, D.M. et al., 2023; Shen et al., 2019).

Table 3. Synergistic Effect of Keratinocyte Regeneration (Wound Healing) by Phyto-DNA and Mineral Mixing Ratio

Mixing Ratio (DNA : Mineral)	Sample 2-1 (1:1)	Sample 2-2 (1:2)	Sample 2-3 (1:5)	Sample 2-4 (1:10)	Mineral Only
Cell Viability (%)	84.7 $\pm$ 5.14	87.5 $\pm$ 4.62	91.2 $\pm$ 5.33	95.7 $\pm$ 6.04	60.3 $\pm$ 3.79

Industrial Prospects as Veterinary Healthcare Materials and Cultured Meat Media

For the rice bran extract and Phyto-PDRN to be applied to the actual bio-industry, biosecurity is the top priority (Handong Co., Ltd. Research Institute, 2025; Heidtmann-Bemvenuti et al., 2012). As a result of ICP-OES analysis, all harmful heavy metals such as lead (Pb), cadmium (Cd), and arsenic (As) were 'not detected (N.D)' in this eco-friendly extract (Table 4) (Handong Co., Ltd. Research Institute, 2025). This is because the 70% ethanol-based extraction process excellently performed the 'selective elution' mechanism, which elutes low-molecular-weight phytochemicals while precipitating high-molecular-weight phytic acid bound to heavy metals (Handong Co., Ltd. Research Institute, 2025).

Table 4. Analysis Results of Harmful Heavy Metals (ICP-OES) Residual Amounts and Raw Material Safety Evaluation of Rice Bran Extract

Test Item (Heavy Metals)	Standard Limit (Feed Additives, etc.)	Measurement Result (mg/kg)	Safety Judgement
Lead (Pb)	≤ 10.0	Not Detected (N.D)	Pass
Arsenic (As)	≤ 2.0	Not Detected (N.D)	Pass
Cadmium (Cd)	≤ 1.0	Not Detected (N.D)	Pass

Synthesizing these high-purity, non-toxic, antioxidant capacity, and cell regeneration data, the complex material derived from rice bran can be developed into impactful biomaterials beyond the limitations of cosmetic patents as follows (Handong Co., Ltd. Research Institute, 2025; Heidtmann-Bemvenuti et al., 2012; Patel & Naik, 2004).

- **Natural PhytoGenics Replacing Antibiotics:** It can function as a feed additive that alleviates intestinal inflammation in livestock caused by high-density rearing or high-temperature stress and promotes beneficial bacteria, thereby replacing AGPs in formula feed and contributing to the improvement of livestock productivity (Gruber et al., 2025; Handong Co., Ltd. Research Institute, 2025).
- **Cultured Meat Material (FBS Alternative):** By supplementing a serum-free media that replaces or reduces expensive fetal bovine serum (FBS), the biggest challenge in the cultured meat industry, with a complex of rice bran PDRN, minerals, and γ -oryzanol as additives, it can establish itself as an innovative technology (IP) that induces the proliferation and differentiation of myoblasts (Handong Co., Ltd. Research Institute, 2025; Patel & Naik, 2004).

IV. CONCLUSION

This study first elucidated the mechanism for mass-producing γ -oryzanol, a core component of rice bran, at a yield (18.5%) and content (7.2 mg/g) exceeding the industrial target through eco-friendly low-temperature reduced-pressure extraction (55°C, 500 mmHg) excluding hexane (Handong Co., Ltd. Research Institute, 2025). In addition, a design platform for a 'dual-functional magnetic affinity bead' that can separate and purify Phyto-PDRN with high purity while excluding complex impurities in rice bran was established, thereby laying the foundation for a process patent (Handong Co., Ltd. Research Institute, 2026a, 2026b; Rice Bran Research Association, 2026). The excellent cytotoxicity mitigation (80.5%) and tissue regeneration synergy through mineral fusion (95.7%) confirmed in the plant Phyto-DNA model support the strong biological activity of this material (Kim, D.M. et al., 2023). In conclusion, it is proven that the rice bran extract platform, perfectly secured with heavy metal safety (N.D), has an overwhelming competitive edge that can innovate the future healthcare and food tech industries as veterinary medicines, phytoGenics feed additives to replace livestock antibiotics, and a new FBS-alternative material for cultured meat (Gruber et al., 2025; Handong Co., Ltd. Research Institute, 2025).

ACKNOWLEDGEMENT

This research was supported by the 'Industry-Academia-Research Collabo R&D (Preliminary Research)' project planned with the financial resources of the Ministry of SMEs and Startups in 2025. We would like to express our deep gratitude to the executives and employees of the Handong Co., Ltd. Research Institute and the officials of the Rice Bran Research Association for their help in successful project planning and optimization of mass production of veterinary healthcare materials.

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