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Biological Patterning in Renal Cell Platforms Following Exposure to a Polyhedral Hydrosol

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ABSTRACT

Chronic kidney disease is marked by gradual functional decline driven by sustained inflammatory signaling, fibrotic remodeling, dysregulated fluid handling, and impaired mitochondrial homeostasis. Although botanical formulations used in traditional health systems are frequently described as kidney-supportive, their cellular-level actions remain insufficiently defined. In this study, a composite botanical hydrosol prepared from *Alpinia oxyphylla* Miq. and fermented plant materials derived from *Polygonatum kingianum*, *Euryale ferox*, and *Lycium chinense* was examined using renal epithelial cell–based experimental systems. The investigation focused on inflammatory modulation, fibrotic pathway activity, aquaporin-related responses, and mitochondrial-associated gene expression.

The hydrosol markedly reduced nitric oxide release in lipopolysaccharide-challenged macrophages, indicating suppression of inflammatory activation. In HEK293 cells exposed to interleukin-1β, treatment produced a temporally distinct immunomodulatory profile, characterized by early elevation of regulatory cytokines (IL-6, IL-8, and IL-10) followed by later-stage downregulation of pro-inflammatory mediators, including IL-12A and interferon-γ. In transforming growth factor-β1–stimulated fibrosis models, hydrosol exposure limited extracellular matrix deposition and maintained epithelial structural integrity. While aquaporin-3 expression exhibited only a modest, non-significant rise, the overall trend suggested supportive regulation of water transport mechanisms.

At the intracellular level, the formulation induced pronounced activation of genes linked to mitochondrial quality control and protein homeostasis. This response included a greater than fourfold increase in *Parkin* expression, along with notable upregulation of *Ubl5*, *NADSYN1*, *Atg8*, and multiple chaperonin complex components (CCT2, CCT6A, and CCT8). Collectively, these findings demonstrate that the botanical hydrosol promotes coordinated anti-inflammatory, anti-fibrotic, and mitochondrial-supportive cellular responses. The results provide mechanistic evidence supporting the development of hydrosol-based nutritional strategies aimed at maintaining renal cellular resilience and promoting healthy aging.

Keywords

Plant-derived hydrosol
Renal cellular models
Inflammatory regulation
Fibrotic signaling
Mitochondrial quality control
Nutritional intervention

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INTRODUCTION

Chronic kidney disease (CKD) has emerged as a major global public health challenge, characterized by a sustained decline in renal functional capacity accompanied by chronic inflammatory activity and progressive fibrotic remodeling. Environmental and lifestyle-related contributors, including excessive sodium consumption and prolonged hyperglycaemic states, play a critical role in accelerating renal injury and disease progression [1]. In parallel, dysregulation of fluid handling, accumulation of senescent renal cells, and compromised aquaporin-mediated water transport mechanisms further aggravate functional deterioration and negatively affect patient well-being [2–4]. Collectively, these pathological disturbances intensify glomerular pressure, disrupt tubular electrolyte reabsorption, impair osmotic balance, and stimulate interstitial matrix expansion, ultimately culminating in end-stage renal disease (ESRD) [5]. Despite the widespread clinical use of pharmacological therapies such as renin–angiotensin system (RAS) blockade, the global incidence of CKD continues to increase, underscoring the need for complementary, non-pharmacological approaches. Population-based studies consistently demonstrate that elevated dietary sodium intake worsens hypertension and proteinuria in CKD populations, while persistent hyperglycaemia remains a dominant etiological factor driving renal damage, particularly in individuals with type 2 diabetes mellitus [6,7].

Within the theoretical framework of traditional Chinese medicine (TCM), renal function is intricately linked to systemic fluid balance, metabolic stability, and aging processes. *Alpinia oxyphylla* Miq., a medicinal–edible botanical formally recorded in the Chinese Pharmacopoeia and traditionally incorporated into classical prescriptions such as *Suoquan* Pill, has long been employed to support kidney vitality, regulate urinary function, and alleviate symptoms including frequent urination, nocturnal emissions, and cold sensations affecting the lower body. Advances in phytochemical research have revealed that *A. oxyphylla* Miq. is rich in biologically active constituents, including volatile oils (such as borneol and camphor), flavonoids, and sesquiterpenes, which collectively exhibit antioxidant, anti-inflammatory, and neuroprotective activities [8,9]. Mechanistic studies have begun to validate these traditional applications at the cellular level. In macrophage and epithelial cell models stimulated with lipopolysaccharide (LPS), extracts of *A. oxyphylla* Miq. significantly attenuate the release of inflammatory mediators, including nitric oxide (NO), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α), primarily through activation of the Nrf2/heme oxygenase-1 (HO-1) signaling axis [10]. In renal injury paradigms, volatile oil fractions from *A. oxyphylla* Miq. have demonstrated protective effects by mitigating oxidative stress and limiting tissue damage in rodent models of cisplatin-induced acute kidney injury [11]. Moreover, specific essential oil components such as camphor and linalool have been associated with smooth muscle relaxation and diuretic activity [12], suggesting potential benefits in relieving urinary retention and edema—hallmark features of early renal dysfunction [11]. Together, these findings provide contemporary scientific support for the traditional classification of *A. oxyphylla* Miq. as a kidney-supportive and fluid-regulating herb.

Beyond *A. oxyphylla* Miq., several botanicals classified under the concept of Medicine–Food Homology (MFH), including *Polygonatum kingianum*, *Euryale ferox*, and *Lycium chinense*, have attracted increasing scientific interest due to their renal-protective, antioxidant, and metabolic-modulating properties. Experimental evidence indicates that *P. kingianum* enhances mitochondrial membrane stability and activates the GSK-3β/Fyn/Nrf2 signaling pathway, while *E. ferox* and *L. chinense* exhibit broad anti-inflammatory effects and support metabolic and mitochondrial homeostasis [13–15]. Importantly, fermentation-assisted processing has been shown to substantially improve the biological activity of these botanicals by breaking down complex polysaccharides into low-molecular-weight oligosaccharides, peptides, and flavonoid glycosides. These transformations enhance cellular bioavailability and promote activation of cytoprotective pathways, including Nrf2 signaling, AMP-activated protein kinase (AMPK), and mitophagy-related mechanisms [16].

Based on these complementary properties, a novel formulation was developed that combines a hydrosol derived from *Alpinia oxyphylla* Miq. with a fermented botanical complex prepared from *P. kingianum*, *E. ferox*, and *L. chinense*. Hydrosol preparations retain both volatile and water-soluble constituents while providing a gentle, biocompatible, and ingestion-friendly delivery format suitable for functional food applications. Given the central involvement of inflammatory signaling, aquaporin dysregulation, fibrotic remodeling, and cellular aging in CKD pathogenesis, we postulated that this integrated formulation could exert coordinated protective actions across multiple renal-relevant pathways.

To evaluate this hypothesis, the present study examined the formulation's capacity to regulate inflammatory responses by quantifying NO production and cytokine gene expression in LPS- and IL-1β-stimulated cellular systems. Cytoprotective effects under osmotic stress were assessed using sodium chloride–challenged Madin–Darby canine kidney (MDCK) cells. Modulation of Aquaporin-3 (AQP3), a critical membrane channel involved in renal water transport and fluid balance, was evaluated to explore potential effects on hydration-related mechanisms.

Anti-fibrotic activity was investigated in transforming growth factor-β1 (TGF-β1)–induced fibrotic models, while impacts on aging-associated cellular processes were assessed through analysis of chaperonin-containing TCP1 (CCT) complex gene expression and regulators of mitochondrial biogenesis. Accordingly, this study aimed to systematically characterize the in vitro effects of a multi-herb botanical hydrosol—comprising *Alpinia oxyphylla* Miq. and fermented MFH botanicals—on renal inflammation, fibrosis, aquaporin regulation, and mitochondrial activation using established epithelial cell models [17]. To the best of our knowledge, this represents the first comprehensive cellular evaluation of a hydrosol-based formulation targeting multiple CKD-associated mechanisms.

MATERIALS AND METHODS

Test formulation and composition

The investigational product used in this work was a composite botanical hydrosol formulated around *Alpinia oxyphylla* Miq. hydrosol as the principal component, supplemented with a fermented botanical blend derived from three Medicine–Food Homology (MFH) plants: *Polygonatum kingianum* Coll. et Hemsl. (rhizome), *Euryale ferox* Salisb. (seed), and *Lycium chinense* Mill. (seed). These plant materials were selected based on their documented inclusion in traditional kidney-supportive formulations described in classical Chinese medical literature and the Chinese Pharmacopoeia. Botanical raw materials were taxonomically authenticated by TCI Co., Ltd. (Taipei, Taiwan). Fermentation of the MFH components was conducted using food-grade microbial strains under controlled environmental conditions, including regulated temperature and pH [18].

In addition to the botanical extracts, the final formulation incorporated plant-derived auxiliary ingredients to support stability and palatability. These included concentrated juices of purple carrot (*Daucus carota*), mulberry (*Morus alba*), and white gourd (*Benincasa hispida*), raspberry (*Rubus idaeus*) powder, pectin, soy lecithin, gum acacia, zinc gluconate, citric acid, sucralose, and purified water. Manufacturing was performed by TCI Co., Ltd., and the finished product was provided by Shanxi Agricultural Valley BaoRenTang Food Co., Ltd. (Shanxi, China). For all in vitro assays, the hydrosol formulation was freshly diluted with sterile water to the required concentrations immediately before application [19].

Cell culture conditions

Three established cell lines were employed to evaluate inflammatory, epithelial, and renal cellular responses: RAW 264.7 murine macrophages (ATCC TIB-71), Madin–Darby canine kidney (MDCK) cells (ATCC CCL-34), and HEK293 human embryonic kidney cells (ATCC CRL-1573). RAW 264.7 cells were maintained in high-glucose Dulbecco's Modified Eagle Medium (DMEM; Gibco, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, USA) and 1% penicillin–streptomycin. MDCK cells were cultured in Minimum Essential Medium (MEM; Gibco, USA) containing 10% FBS, 1% penicillin–streptomycin, and 1 mM sodium pyruvate. HEK293 cells were propagated in DMEM supplemented with 10% FBS and 1% antibiotic–antimycotic solution (Gibco, USA). All cell cultures were maintained at 37 °C in a humidified incubator with 5% CO_2.

Assessment of nitric oxide production

Anti-inflammatory activity was evaluated by quantifying nitric oxide (NO) production in RAW 264.7 macrophages using the Griess reaction. Cells were seeded into 96-well plates at a density of 1×10^4 cells per well and allowed to adhere for 24 h. Inflammatory stimulation was induced by exposure to lipopolysaccharide (LPS; 200 ng/mL; Sigma-Aldrich, USA) in serum-free DMEM, either in the presence or absence of the hydrosol formulation (0.0625% v/v), for an additional 24 h. Culture supernatants were subsequently collected and reacted with freshly prepared Griess reagent (Invitrogen™ G-7921, Thermo Fisher Scientific, USA). Following a 30-min incubation at room temperature, absorbance was measured at 548 nm using a microplate reader (Epoch™, BioTek, USA). NO levels were normalized to the LPS-treated control group, which was defined as 100%.

Evaluation of cytoprotection under hyperosmotic stress

The protective effects of the hydrosol against osmotic stress were examined in MDCK cells. Cells were seeded into 96-well plates at a density of 3×10^3 cells per well and cultured overnight. Hyperosmotic conditions were established by adjusting MEM to 500 mOsm/kg using sodium chloride. Cells were then exposed to hyperosmotic medium with or without hydrosol supplementation (0.0625% v/v) for 48 h [20]. Cell viability was assessed using an MTT assay. Following treatment, cells were incubated with MTT solution (5 mg/mL; Sigma-Aldrich, USA) for 2–3 h. The medium was removed, and the resulting formazan crystals were solubilized in dimethyl sulfoxide (DMSO). Absorbance was measured at 570 nm, and cell viability was expressed as a percentage relative to isotonic control conditions.

Immunofluorescent analysis of Aquaporin-3 expression

Regulation of renal water transport was examined by

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assessing Aquaporin-3 (AQP3) expression in MDCK cells using immunofluorescence staining. Cells were seeded into 24-well plates at a density of 2×10^4 cells per well and treated with or without hydrosol (0.125% v/v) for 24 h. Following treatment, cells were fixed with 10% formaldehyde, permeabilized using 0.5% Triton X-100, and blocked with 1% bovine serum albumin (BSA). Cells were incubated overnight at 4 °C with a primary anti-AQP3 antibody (1:1000; Boster, USA), followed by incubation with an Alexa Fluor 488-conjugated secondary antibody (1:2000; Thermo Fisher Scientific, USA) for 1 h at 37 °C. Nuclear counterstaining was performed using Hoechst 33342. Fluorescence images were captured using an inverted fluorescence microscope (Axio Vert.A1, ZEISS, Germany), and quantitative analysis of mean fluorescence intensity was conducted with ImageJ software.

TGF-β1-induced fibrotic model

Antifibrotic activity was evaluated using a transforming growth factor-β1 (TGF-β1)–induced fibrosis model in MDCK cells. Cells were seeded into 24-well plates at a density of 2×10^4 cells per well and exposed to TGF-β1 (5 ng/mL; Sigma-Aldrich, USA) in the presence or absence of hydrosol treatment (0.0625% v/v) for 48 h. After treatment, cells were fixed, permeabilized, and stained with ActinRed™ 555 (Thermo Fisher Scientific, USA) to visualize cytoskeletal organization. Nuclei were counterstained with Hoechst 33342. Morphological changes associated with fibrotic transformation were qualitatively assessed by fluorescence microscopy.

Quantitative real-time PCR analysis

Gene expression analyses were performed in HEK293 cells to evaluate inflammatory and aging-related molecular responses. For inflammatory assessments, cells were treated with recombinant interleukin-1β (IL-1β; 10 ng/mL; PeproTech, USA) in the presence or absence of hydrosol (0.125% v/v) for 6 h or 24 h. For aging-related analyses, cells were treated with hydrosol alone (0.125% v/v) for 48 h. Total RNA was isolated using a commercial extraction kit (Geneaid, Taiwan), and 2,000 ng of RNA was reverse-transcribed using SuperScript® III Reverse Transcriptase (Invitrogen, USA). Quantitative PCR was conducted with KAPA SYBR® FAST qPCR Master Mix (KAPA Biosystems, USA) on a StepOnePlus™ Real-Time PCR System (Applied Biosystems, USA).

Target genes for inflammatory responses included *IL-6*, *IL-8*, *IL-10*, *IL-12A*, and *IFN-γ* for primary analyses, with *IL-3*, *IL-18*, and *IL-23* evaluated in supplementary experiments. Aging-related targets included *CCT2*, *CCT5*, *CCT6A*, *CCT7*, *CCT8*, *Parkin*, *Atg8*, *NADSYN*, and *Ubl5*. *GAPDH* was used as the internal normalization control. Primer sequences are provided in Table 1. All primers were designed and validated using Primer-BLAST (NCBI) and synthesized by Genomics (New Taipei City, Taiwan). Relative gene expression levels were calculated using the $2^{-}\Delta\Delta Ct$ method [21].

Statistical analysis

Experimental data are presented as mean ± standard deviation (SD). Statistical comparisons between groups were performed using Student's *t*-test implemented in Microsoft Excel (USA). Differences were considered statistically significant at $p < 0.05$.

Table 1: Summary of Multi-Herb Botanical Hydrosol Effects on Renal Cell Models.

Experimental Model	Treatment / Concentration	Observed Effects	Key Findings / Quantitative Data
RAW 264.7 macrophages	LPS ± Hydrosol (0.0625%)	Anti-inflammatory	NO production reduced to 85.7 ± 3.9% vs. LPS-only (100%) (p<0.05)
MDCK cells (hyperosmotic stress)	NaCl ± Hydrosol (0.0625%)	Cytoprotection	Cell viability partially restored: 58.1 ± 21.0% vs. 46.5 ± 0.0% (NaCl only)
MDCK cells (AQP3 expression)	Hydrosol (0.125%)	Water channel regulation	Mean fluorescence intensity increased 5.4% (105.4 ± 7.0% vs. 100 ± 5.0%, p=0.34)
MDCK cells (TGF-β1-induced fibrosis)	TGF-β1 ± Hydrosol (0.0625%)	Anti-fibrotic	Reduced ECM deposition; more organized cytoskeleton resembling control
HEK293 cells (IL-1β stimulation, 6h)	Hydrosol (0.125%)	Anti-inflammatory cytokines	IL-6: 1.79 ± 0.20 (p<0.01), IL-8: 1.50 ± 0.18 (p<0.05), IL-10: 1.45 ± 0.32 (p<0.05)
HEK293 cells (IL-1β stimulation, 24h)	Hydrosol (0.125%)	Pro-inflammatory cytokine suppression	IL-12A: 0.64 ± 0.06, IFN-γ: 0.61 ± 0.03 (p<0.05)
HEK293 cells (48h)	Hydrosol (0.125%)	Anti-aging / mitochondrial activation	Upregulation: CCT2 (p<0.05), CCT6A & CCT8 (p<0.01), Parkin >4-fold (p<0.01), Ubl5 (p<0.001), NADSYN (p<0.01), Atg8 (p<0.05)

RESULTS

Suppression of nitric oxide production by the botanical hydrosol

The anti-inflammatory activity of the botanical hydrosol was first examined by measuring nitric oxide (NO) release in lipopolysaccharide (LPS)-activated RAW 264.7 macrophages. As expected, LPS stimulation markedly increased NO production relative to unstimulated cells, which were defined as the baseline control (100%) (Figure 1). Exposure to the botanical hydrosol at 0.0625% significantly reduced NO levels to 85.7 ± 3.9% of the LPS-treated group ($p < 0.05$, $n = 3$). Interestingly, NO production in hydrosol-treated cells was marginally lower than that observed in the untreated control group (87.9 ± 2.1%), indicating a pronounced inhibitory effect on inflammatory nitric oxide synthesis [22].

Protective trend of the botanical hydrosol under hyperosmotic conditions

To determine whether the botanical hydrosol could mitigate osmotic stress–induced cytotoxicity, MDCK cells were exposed to elevated sodium chloride concentrations (+50 mM) for 24 h in the presence or absence of hydrosol supplementation. Hyperosmotic challenge resulted in a substantial reduction in cell viability, decreasing to 46.5 ± 0.0% compared with isotonic controls (100 ± 5.2%, $p < 0.001$), as illustrated in Figure 2. Co-treatment with the botanical hydrosol increased cell viability to 58.1 ± 21.0%. Although this improvement did not reach statistical significance ($p = 0.393$), the observed increase suggests a partial protective effect against salt-induced cellular injury, with notable variability among replicates.

Modest enhancement of Aquaporin-3 expression following hydrosol exposure

The influence of the botanical hydrosol on renal water transport mechanisms was evaluated by examining Aquaporin-3 (AQP3) expression in MDCK cells using immunofluorescence analysis. After 24 h of treatment with 0.125% hydrosol, cells displayed a visible increase in AQP3-associated green fluorescence compared with untreated controls (Figure 3). Quantitative assessment of mean fluorescence intensity (MFI) demonstrated a 5.4% elevation in the hydrosol-treated group relative to controls (105.4 ± 7.0% vs. 100.0 ± 5.0%; $p = 0.340$). While this increase was not statistically significant, the trend suggests a potential modulatory effect of the formulation on aquaporin expression.

Attenuation of TGF-β1-driven fibrotic alterations

The anti-fibrotic capacity of the botanical hydrosol was investigated using a transforming growth factor-β1 (TGF-β1)-induced fibrosis model in MDCK cells. Treatment with

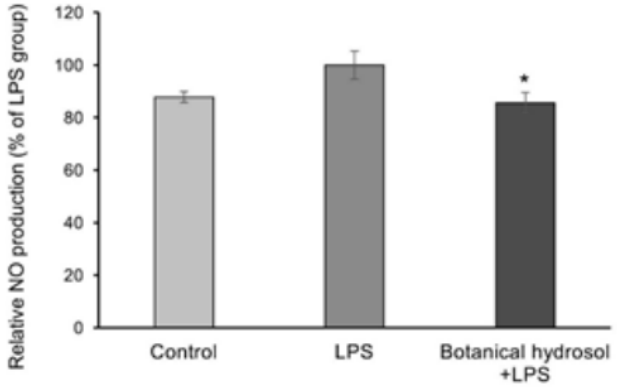

Figure 1: Inhibitory effect of the botanical hydrosol on nitric oxide production in LPS-stimulated macrophages.

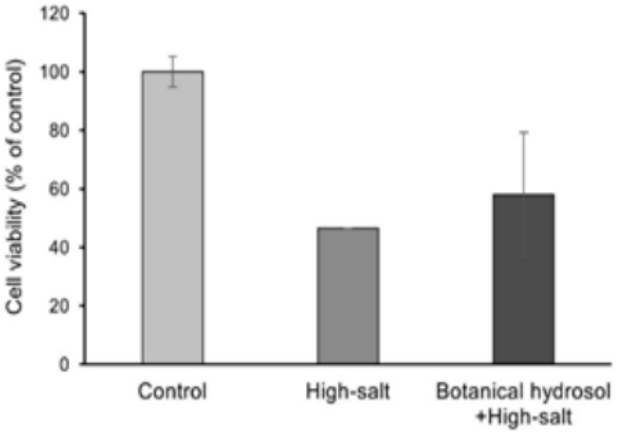

Figure 2: Cytoprotective effect of the botanical hydrosol against high-salt-induced cell stress.

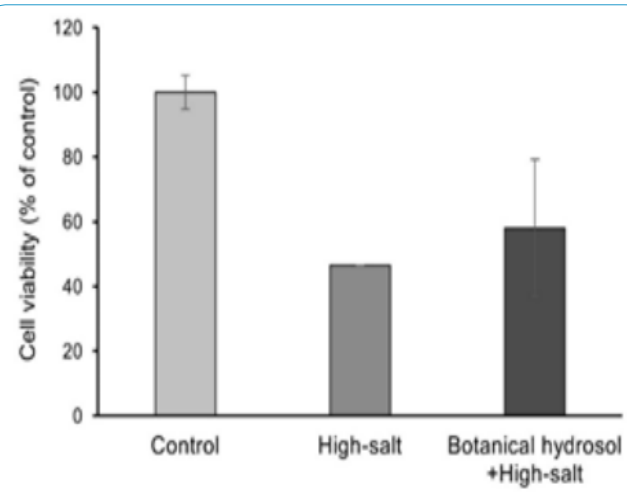

Figure 3: Effect of botanical hydrosol on aquaporin-3 expression in MDCK cells.

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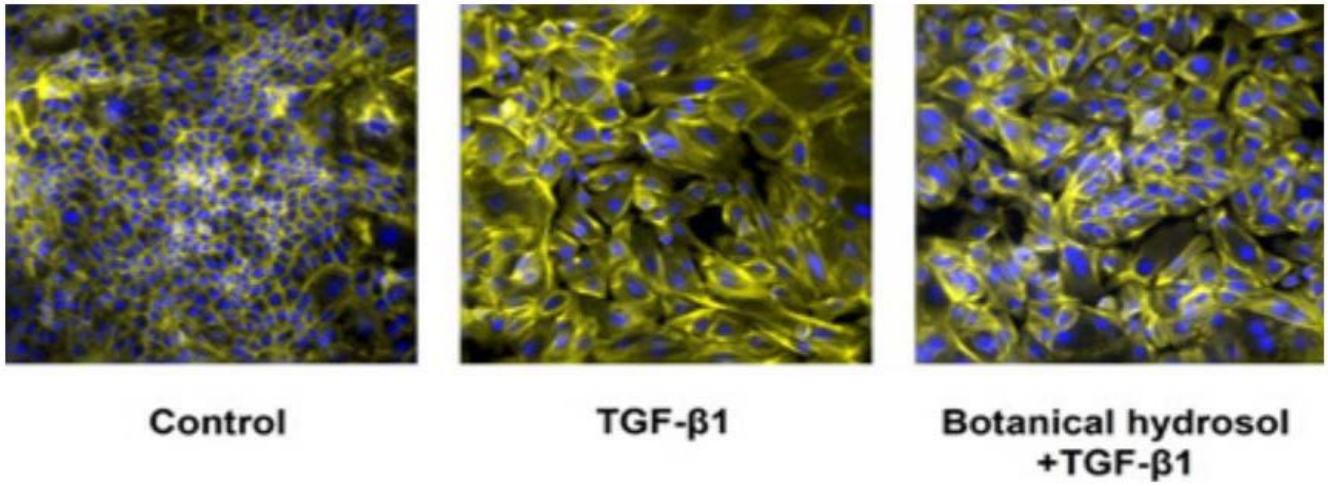

Figure 4: Anti-fibrotic effect of botanical hydrosol in a TGF-β1-induced renal fibrosis model.

TGF-β1 (5 ng/mL) for 48 h resulted in pronounced fibrotic features, including enhanced extracellular matrix deposition and the appearance of dense, fiber-like structures, as visualized by immunofluorescence staining (Figure 4). In contrast, cells co-treated with the botanical hydrosol exhibited a visibly reduced fibrotic phenotype, characterized by a more organized cytoskeletal architecture and diminished matrix accumulation. The morphological profile of these cells more closely resembled that of untreated controls, indicating that the hydrosol may counteract TGF-β1-mediated fibrotic remodeling in renal epithelial cells [23].

Temporal modulation of inflammatory cytokine gene expression

To further characterize the immunomodulatory properties of the botanical hydrosol, cytokine gene expression was analyzed in HEK293 cells stimulated with interleukin-1β (IL-1β). At the early 6 h time point, co-treatment with 0.125% hydrosol significantly enhanced the expression of cytokines associated with regulatory or compensatory inflammatory responses, including IL-6 (1.79 ± 0.20, $p < 0.01$), IL-8 (1.50 ± 0.18, $p < 0.05$), and IL-10 (1.45 ± 0.32, $p < 0.05$), compared with IL-1β stimulation alone. This early response suggests an active immunoregulatory adjustment during acute inflammatory challenge.

At 24 h, a distinct shift in cytokine expression was observed. The expression of pro-inflammatory mediators IL-12A and interferon-γ (IFN-γ) was significantly reduced in the hydrosol-treated group (0.64 ± 0.06 and 0.61 ± 0.03, respectively; $p < 0.05$), indicating suppression of sustained inflammatory signaling. Additional cytokines, including IL-3, IL-18, and IL-23, exhibited downward trends at this later time point, although these changes did not reach statistical significance. Together, these findings suggest that the botanical hydrosol exerts a time-dependent regulatory effect on inflammatory gene expression.

Activation of chaperonin and mitochondrial-associated gene networks

To explore potential effects on cellular aging and mitochondrial homeostasis, the expression of chaperonin-containing TCP1 complex (CCT) genes and mitochondrial-related markers was assessed in HEK293 cells following 48 h of hydrosol exposure. Treatment with the botanical hydrosol resulted in significant upregulation of several CCT family members, including *CCT2* ($p < 0.05$), *CCT6A*, and *CCT8* ($p < 0.01$), while *CCT5* and *CCT7* displayed non-significant upward trends.

In parallel, genes involved in mitochondrial quality control and bioenergetic regulation were strongly induced. Expression of *Parkin* increased by more than fourfold ($p < 0.01$), accompanied by significant elevations in *Ubl5* ($p < 0.001$), *NADSYN* ($p < 0.01$), and *Atg8* ($p < 0.05$). These coordinated transcriptional changes suggest that the botanical hydrosol supports mitochondrial function and proteostasis through activation of aging-related gene networks.

DISCUSSION

Chronic kidney disease (CKD) arises from the convergence of immune imbalance, persistent fibrotic remodeling, dysregulated water handling, and progressive mitochondrial impairment. Rather than operating as isolated defects, these processes reinforce one another, accelerating renal functional decline. In this context, the present in vitro study demonstrates that a multi-herb botanical hydrosol—comprising *Alpinia*

oxyphylla Miq. and fermented extracts of *Polygonatum kingianum*, *Euryale ferox*, and *Lycium chinense*—exerts broad regulatory effects across several pathways directly implicated in CKD pathophysiology. These effects include suppression of inflammatory nitric oxide production, temporal modulation of cytokine expression, attenuation of profibrotic responses, modest regulation of aquaporin expression, and activation of mitochondrial and proteostasis-related gene networks [24].

Alpinia oxyphylla Miq. has a long-standing role in traditional Chinese medicine, where it is prescribed to restore kidney "yang," regulate urination, and stabilize fluid balance. Contemporary pharmacological investigations attribute many of its bioactivities to volatile constituents such as borneol and camphor, which have been shown to activate cytoprotective signaling cascades including the Nrf2/HO-1 axis. The significant reduction in nitric oxide production observed in our macrophage model aligns with these reports and supports an anti-inflammatory mechanism consistent with oxidative stress modulation. Moreover, the early elevation of IL-6 and IL-10 expression suggests that the hydrosol may not merely suppress inflammation, but actively promote a regulated immune response that favors resolution rather than chronic activation [25].

Beyond *A. oxyphylla*, the inclusion of fermented *P. kingianum*, *E. ferox*, and *L. chinense* likely contributed to the breadth of biological responses observed. These botanicals belong to the Medicine–Food Homology (MFH) category, reflecting their dual role as nutritional and therapeutic agents. *Polygonatum* species are traditionally associated with kidney and essence nourishment and are rich in polysaccharides, saponins, and homoisoflavonoids—compounds known to enhance antioxidant defenses, stabilize mitochondrial function, and modulate immune signaling. *E. ferox* contains polyphenolic and alkaloid constituents with documented anti-inflammatory and diuretic effects, while *L. chinense* has been reported to influence mitochondrial energy metabolism and cellular stress resistance, in part through betaine-mediated pathways. Importantly, fermentation is known to enzymatically convert complex macromolecules into low-molecular-weight metabolites with enhanced bioavailability, potentially amplifying cellular uptake and signaling efficacy. Such fermentation-derived metabolites may act in concert to activate protective pathways such as AMPK, Nrf2, and mitophagy-associated signaling, thereby producing a synergistic rather than additive biological effect [26-29].

Inflammation and fibrosis represent central drivers of CKD progression, often sustained by prolonged cytokine signaling and oxidative injury. Our data extend existing knowledge by revealing a time-dependent immunological modulation. During the early inflammatory phase, the botanical hydrosol enhanced expression of IL-6, IL-8, and IL-10—cytokines increasingly recognized for their roles in immune regulation, epithelial repair, and inflammation resolution. In contrast, prolonged stimulation revealed suppression of IL-12A and IFN-γ, suggesting dampening of Th1-associated inflammatory signaling. This biphasic response implies that the formulation may recalibrate immune dynamics rather than uniformly suppress immune activity, potentially influencing macrophage polarization or antigen-presenting cell behavior in a manner conducive to tissue protection [30].

Consistent with its immunomodulatory profile, the botanical hydrosol also mitigated TGF-β1–induced fibrotic remodeling in renal epithelial cells. TGF-β1 is widely recognized as a master regulator of renal fibrosis, driving SMAD-dependent transcription of extracellular matrix components and promoting epithelial-to-mesenchymal transition (EMT). The marked reduction in extracellular matrix accumulation and preservation of epithelial morphology observed with hydrosol co-treatment suggest interference with profibrotic signaling. This effect may involve antioxidant-mediated attenuation of TGF-β1 signaling or disruption of downstream EMT pathways, both of which are closely linked to redox-sensitive regulatory nodes such as Nrf2, AKT, and HO-1. Collectively, these findings support a dual anti-inflammatory and anti-fibrotic role for the hydrosol formulation.

Water homeostasis is another critical yet often underappreciated aspect of CKD pathogenesis. Aquaporin-3 (AQP3), abundantly expressed in renal collecting ducts, facilitates transcellular water transport and contributes to osmotic equilibrium. Reduced AQP3 expression has been associated with impaired urinary concentrating capacity and interstitial fluid imbalance in early renal dysfunction. In the present study, hydrosol treatment elicited a modest, though non-significant, increase in AQP3 expression. While preliminary, this trend may reflect early transcriptional priming or membrane-stabilizing effects. Phytochemical constituents such as polysaccharide derivatives and flavonoids—reported in analogous botanical systems to modulate aquaporin expression via cAMP-PKA or PI3K-Akt signaling—may underlie this observation. Further investigation using extended exposure periods, dose escalation, and protein-level analyses will be necessary to clarify this potential regulatory mechanism [31].

Emerging evidence identifies mitochondrial dysfunction and loss of proteostasis as central mechanisms underlying renal aging and CKD progression. In this study, the botanical hydrosol markedly upregulated multiple members of the chaperonin-containing TCP1 (CCT/TRiC) complex, including *CCT2*, *CCT6A*, and *CCT8*. These chaperonins are essential for

correct folding of cytoskeletal and stress-sensitive proteins, maintenance of proteome integrity, and cellular recovery following injury. Decline in CCT expression has been linked to aging-related proteotoxic stress, whereas preservation of CCT function supports cellular resilience. Given the reliance of renal epithelial cells on cytoskeletal integrity for barrier function and repair, activation of CCT-mediated folding machinery may represent a critical protective mechanism.

In parallel, robust activation of mitochondrial quality control pathways was observed. The pronounced induction of *Parkin*—a key regulator of PINK1-Parkin–dependent mitophagy—suggests enhanced clearance of damaged mitochondria. Parkin-mediated mitophagy has been shown to limit tubular injury, suppress inflammasome activation, and preserve renal function in multiple experimental models of kidney damage. Upregulation of *NADSYN1*, *Ubl5*, and *Atg8* further supports coordinated enhancement of mitochondrial biogenesis, autophagic flux, and redox homeostasis. Together, these transcriptional changes point toward a rejuvenation-oriented response aimed at restoring mitochondrial integrity and cellular energy balance.

Notably, increased *Ubl5* expression may indicate activation of the mitochondrial unfolded protein response (UPRmt), a conserved stress adaptation pathway essential for maintaining mitochondrial proteostasis. Ubl5 functions as a critical co-factor in UPRmt signaling, and its deficiency has been associated with accelerated aging and heightened vulnerability to metabolic stress. Recent studies implicate ATF5–Ubl5–mediated UPRmt activation in protection against tubular injury in diabetic nephropathy, suggesting that reinforcement of this pathway may enhance renal stress tolerance. The observed upregulation of Ubl5 in response to the botanical hydrosol therefore provides further mechanistic support for its role in promoting mitochondrial resilience.

In summary, this study demonstrates that a fermented multi-herb botanical hydrosol exerts coordinated anti-inflammatory, anti-fibrotic, and mitochondrial-supportive effects across multiple CKD-relevant pathways. Rather than targeting a single molecular axis, the formulation appears to engage an integrated network of immune regulation, proteostasis maintenance, and mitochondrial quality control. These findings provide mechanistic justification for further exploration of hydrosol-based functional foods as supportive interventions for renal health and healthy aging.

CONCLUSION

The present investigation demonstrates that a fermented, multi-botanical hydrosol incorporating *Alpinia oxyphylla* Miq. and selected Medicine–Food Homology herbs exerts coordinated protective effects across multiple renal cell–relevant pathways. In cellular models, the formulation dampened inflammatory signaling while inducing a temporally regulated cytokine profile consistent with inflammation resolution and tissue-supportive responses. Concurrently, it mitigated TGF-β1–driven fibrotic alterations in renal epithelial cells and exhibited a modest tendency to influence aquaporin-3 expression, suggesting a potential role in maintaining epithelial integrity and water-handling mechanisms.

At the transcriptional level, the hydrosol robustly activated gene networks involved in mitochondrial surveillance, proteostasis, and cellular stress adaptation. Upregulation of mitophagy-associated markers (*Parkin*, *Atg8*), mitochondrial metabolic regulators (*NADSYN1*), unfolded protein response mediators (*Ubl5*), and key chaperonin complex subunits (*CCT2*, *CCT6A*, *CCT8*) indicates enhancement of mitochondrial quality control, protein-folding capacity, and intracellular homeostasis. These coordinated molecular responses suggest that the formulation supports cellular resilience mechanisms that are increasingly recognized as critical determinants of renal aging and chronic kidney vulnerability. Collectively, the findings support the concept that botanical hydrosols—particularly those derived from fermented, kidney-associated medicinal plants—may function as multi-target biological modulators rather than single-pathway agents. While the current work is confined to in vitro systems, it establishes a mechanistic foundation for future studies. Validation in animal models, alongside protein-level and functional assessments, will be essential to determine physiological relevance and evaluate the translational potential of such formulations for kidney health maintenance and healthy aging strategies.

CONFLICT OF INTEREST

Authors declare there is no conflict of interest.

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