Evaluation of the Modulatory Effect of Timeless Radiance-Anti-Aging Cream on AQP3 Gene Expression in Human Dermal Fibroblasts (HDF) Cell Line

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***Abstract*—Skin aging is a multifactorial process driven by intrinsic cellular senescence and extrinsic stressors such as ultraviolet (UV) radiation, oxidative stress, and pollution. A hallmark of aged skin is impaired hydration, closely linked to reduced expression of Aquaporin-3 (AQP3), a membrane channel in keratinocytes responsible for transporting water and glycerol. Enhancing AQP3 activity is therefore a promising strategy for maintaining epidermal hydration and delaying visible signs of aging. Herbal formulations have shown potential to modulate hydration, barrier repair, and oxidative stress defence. This study investigated the effect of *Timeless Radiance Anti-Aging Cream* on AQP3 gene expression in human dermal fibroblasts (HDFs). The cytotoxicity of *Timeless Radiance Anti-Aging Cream* was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on HDFs across concentrations ranging from 1000 µg/mL to 7.8 µg/mL. AQP3 mRNA expression was quantified by Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) following treatment with the formulation for 24 hours. GAPDH was used as an endogenous control. The formulation demonstrated no significant cytotoxicity, with an IC_{50} > 1000 µg/mL. qRT-PCR analysis revealed a 1.72 ± 0.15-fold upregulation of AQP3 expression in HDFs treated with 500 µg/mL of the cream compared to untreated controls. This upregulation indicates improved water and glycerol transport potential, suggesting enhanced hydration and barrier function. *Timeless Radiance Anti-Aging Cream* significantly upregulated AQP3 expression without cytotoxic effects, supporting its role in promoting skin hydration and anti-aging benefits. These findings provide molecular evidence for the efficacy of herbal cosmeceuticals as safe and multifunctional interventions for delaying skin aging.**

***Index Terms*—Anti-aging cream, Aquaporin-3, dermal fibroblasts, gene expression, skin hydration**

I. INTRODUCTION

Aging is a natural biological process characterized by the progressive decline of physiological functions, largely driven by the accumulation of molecular and cellular damage. The skin, as the body's outermost protective barrier, is particularly susceptible to both intrinsic and extrinsic aging. Clinically, aged skin exhibits dryness, thinning, wrinkles, reduced elasticity, and delayed cell turnover. Intrinsic contributors include reactive oxygen species (ROS), DNA mutations, telomere shortening, and cellular senescence, whereas extrinsic aggressors like ultraviolet (UV) rays, pollution, irritants, and tobacco smoke accelerate degenerative changes.

The structural hallmarks of skin aging include collagen degradation, elastin breakdown, immune dysfunction, and the accumulation of advanced glycation end products (AGEs), which link oxidative and metabolic stress to dermal deterioration. Over the past two decades, research has focused on molecular targets to delay or reverse skin aging, leading to the rise of cosmeceuticals and minimally invasive therapies. Antiaging combating strategies are commonly classified as primary, aimed at preventing early signs, or secondary, addressing visible aging features already established. [1]

Healthy skin needs a lot of water. Hyaluronic acid (HA) is abundant in the dermis and contributes to moisture content, skin atrophy, and the spread of soluble factors and nutrients. Aquaporin-3 (AQP3) has emerged as a critical regulator of epidermal hydration, elasticity, and repair. AQP3, a membrane channel expressed in keratinocytes, facilitates the transport of water, glycerol, and small solutes. Its function supports not only hydration but also energy

metabolism, barrier recovery, and collagen synthesis. Reduced AQP3 expression is associated with aged or photo-damaged skin, resulting in impaired moisture balance, increased oxidative stress, and accelerated visible aging. [2]

A recent study reveals the potential of natural bioactive and herbal formulations in regulating AQP3 expression and skin health. Herbal creams, enriched with plant-derived antioxidants, polyphenols, and phyto-active compounds, offer a dual approach by protecting against oxidative stress and restoring hydration. Phytocompounds from herbs, such as aloe vera, green tea, licorice, and ginseng, have demonstrated the ability to enhance skin moisture, stimulate collagen synthesis, and reduce inflammation, thereby delay aging. Importantly, phytochemicals may upregulate AQP3 activity, positioning herbal creams as promising, non-invasive interventions in targeted antiaging therapies. [3]

Taken together, integrating molecular insights on AQP3 with the therapeutic potential of herbal creams provides a valuable foundation for the development of safe, effective, and naturally inspired antiaging strategies.

II. MATERIALS AND METHODS

1.1. Objective:

Evaluation of the modulatory effect of *Timeless Radiance-Anti-Aging Cream* on AQP-3 gene expression in human dermal fibroblast cell line

1.2. Outline of the method:

The *in vitro* gene expression was performed for the *Timeless Radiance-Anti-Aging Cream* on Human dermal fibroblast (HDF) cells to evaluate its effect on the AQP3 gene.

1.3. Preparation of *Timeless Radiance-Anti-Aging Cream*

Test product *Timeless Radiance-Anti-Aging Cream* is composed of natural ingredients like Reishi mushroom extract, Frankincense hydrosol, Saffron oil, Sweet Almond oil, and Aloe vera.

10mg of *Timeless Radiance-Anti-Aging Cream* was weighed and dissolved in medium supplemented with 2% inactivated FBS to obtain a stock solution of 10 mg/mL. Furthermore, serial two-fold dilutions were prepared from the stock solution to prepare lower concentrations for cytotoxicity testing.

1.4. Cell line and culture medium

Human dermal fibroblast cell line was procured from American Type Culture Collection (ATCC). Stock cells were cultured in Dulbecco's Modified Eagle Medium, High Glucose (DMEM-HG) supplemented with 10% inactivated Fetal Bovine Serum (FBS), Penicillin (100 IU/mL), Streptomycin (100 µg/mL) and Amphotericin B (5 µg/mL) in a humidified atmosphere of 5% CO_2 at 37 °C until confluent. The cells were dissociated with TE solution (0.2% Trypsin, 0.02% EDTA). The stock cultures were grown in 25 cm^2 culture flasks and cytotoxicity studies were carried out in 96-well microtiter plate.

1.5. Determination of cell cytotoxicity by MTT Assay

The cell culture monolayer was trypsinized, and the cell count was adjusted to 100,000 cells/mL using DMEM-HG containing 10% FBS. To each well of the 96-well microtiter plate, 0.1mL of the diluted cell suspension was seeded into each well. After 24hrs, when a partial monolayer was formed, the supernatant was flicked off, and the monolayer was washed once with Dulbecco's Phosphate Buffered Saline (DPBS). Then cells were treated with different test concentrations varying from 1000µg/mL to 7.8µg/mL. The untreated cells were maintained as a cell control for comparison. The plates were then incubated at 37 °C for 24hrs in 5% CO_2 incubator, and microscopic examination was carried out. After 24hrs, the test solutions in the wells were discarded and 100µL of MTT diluted with DPBS was added to each well. The plate was incubated for 3hrs at 37°C in 5% CO_2 incubator. The supernatant was removed, and 100µL of Dimethyl sulfoxide (DMSO) was added to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 570 nm.

1.6. *In vitro* Gene expression

Human dermal fibroblast (HDF) cells were cultured in 10% growth media (DMEM-HG) supplemented with FBS at 5% CO_2 and 37ºC in a flask. 2 mL of cell suspension was seeded in a 6-well plate at a density of $1x10^5$ cells/mL. When the cells attained 70% confluence, these cells were treated with *Timeless Radiance-Anti-Aging Cream* and incubated for 24hrs. After incubation, cells were subjected to cell lysis by treating with Tri-extract reagent, and chloroform was added to isolate the total RNA from the sample and

subjected to centrifugation. Out of the three distinct layers observed, the upper layer was collected in a fresh tube and an equal volume of isopropanol was added and incubated at -20 °C for 10mins. After the incubation, followed by centrifugation, an appropriate volume of ethanol was added to resuspend the pellet. The resuspended pellet was subjected to centrifugation. The pellet obtained was air-dried, and an appropriate volume of TAE buffer was added. The isolated total RNA was further used for cDNA synthesis. cDNA was synthesized using oligo dT primers followed by reverse transcriptase enzyme treatment according to manufacturer's protocol. The cDNA thus synthesized was used for PCR for the amplification of AQP3 and GAPDH (Internal control).

1.7. Analysis of mRNA expression by quantitativeReal Time-PCR (qRT-PCR)

The mRNA expression levels of AQP3 genes were measured by qRT-PCR using the CFX Opus 96 Real-time PCR system, which directly detects the RT-PCR products without downstream processing. This is achieved by monitoring the increase in fluorescence of a dye-labeled DNA probe, in which one is specific for the gene of interest, and another is specific for the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene, which is used as an endogenous assay control. 20µL of the reaction mixture was subjected to PCR for amplification of genes by using cDNA and specifically designed primers. The endogenous control probe, specific for the GAPDH gene, served to standardize the amount of RNA samples.

1.8. Amplification conditions

The 40 cycles of two-step PCR reactions consist of 5 minutes at 95 °C followed by 35 cycles of denaturation at 95 °C for 30 seconds, annealing at Tm for 30 seconds, and extension at 72 °C for 45 seconds. This was followed by a final extension at 72°C for 10 min.

Primer used: Gene-specific primers were designed for GAPDH using NCBI primer blast and procured from Eurofins, India.

Gene	Primer Sequence (5′–'3)
GAPDH	F- GTCTCCTCTGACTTCAACAGCG R-ACCACCCTGTTGCTGTAGCCAA
AQP-3	F- GCTGTCACTCTGGCATCCTG R- GCGTCTGTGCCAGGGTGTAG

III. RESULTS

The *Timeless Radiance-Anti-Aging Cream,* evaluated for in vitro cytotoxicity on Human Dermal Fibroblasts (HDF) across a concentration range of 1000 µg/mL to 7.8 µg/mL, showed cell growth inhibition. The IC_{50} value was greater than 1000 µg/mL, indicating no significant cytotoxicity on HDF cells.

Quantitative real-time PCR using gene-specific primers revealed a modulatory upregulation of AQP3 mRNA levels in treated cells compared with untreated controls.

Table I: In vitro cytotoxicity of *Timeless Radiance-Anti-Aging Cream* in terms of percentage cell viability against Human dermal fibroblast (HDF) cell line by MTT assay (24 Hours).

Concentration (µg/mL)	Percentage of cell viability (Mean ± SD)	CTC 50 (µg/mL)
1000	84.31 ± 0.89	2410.233
500	86.34 ± 0.57	
250	92.01 ± 1.09	
125	94.82 ± 2.98	
62.5	97.33 ± 3.39	
31.25	98.66 ± 4.63	
15.625	99.59 ± 1.60	
7.8	102.69 ± 0.84	

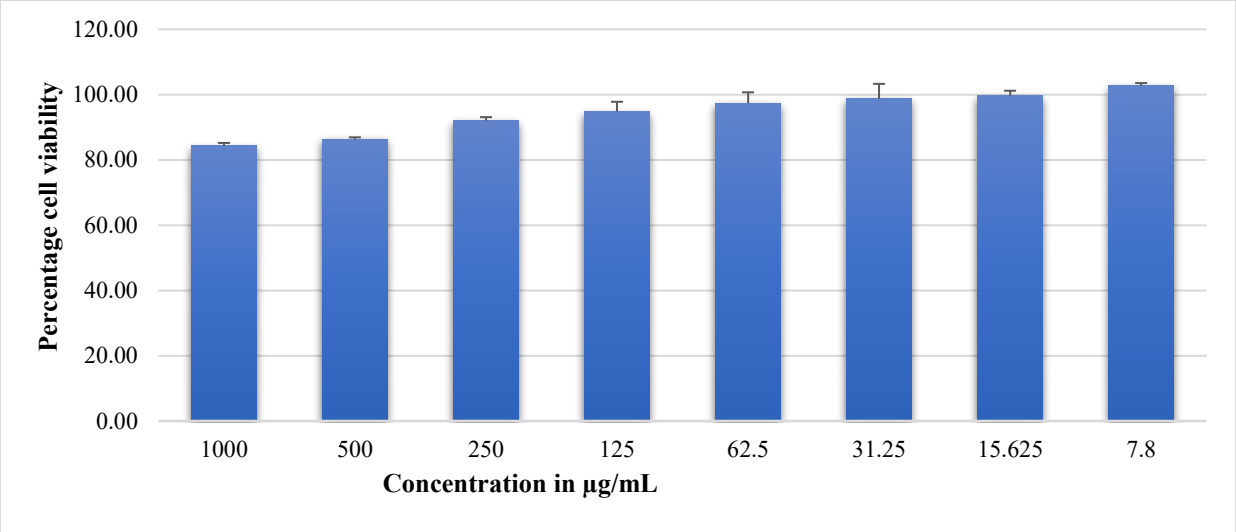

Fig. I: Effect of *Timeless Radiance-Anti-Aging Cream* in terms of percentage cell viability on HDF cell line

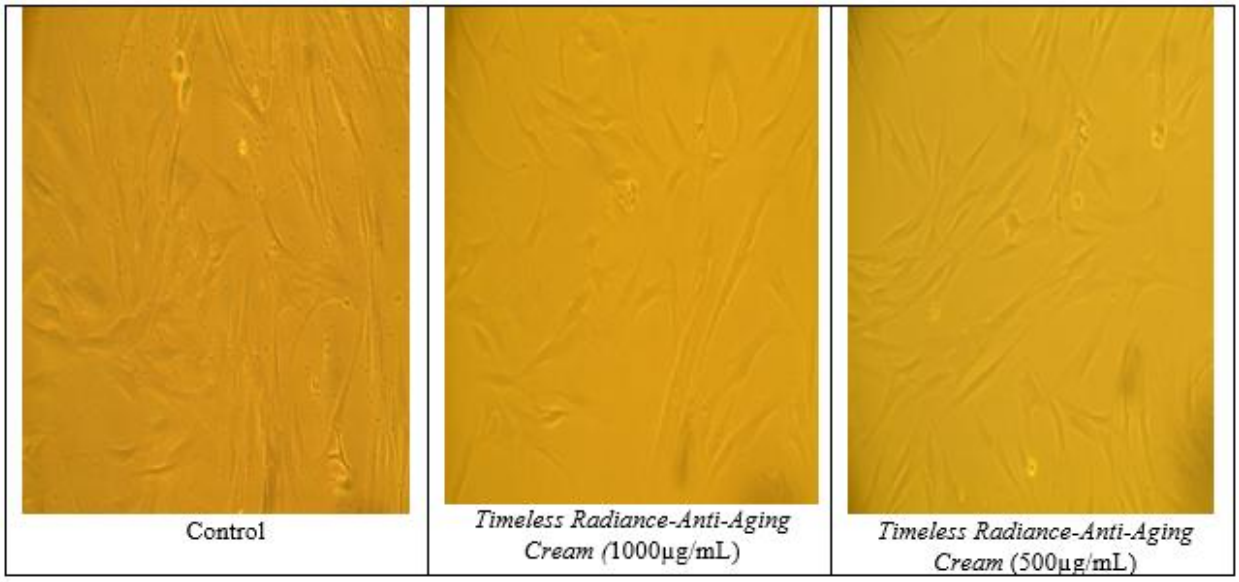

Fig. II: Microscopic images showing cell cytotoxicity at 24 hrs. in cells treated with *Timeless Radiance-Anti-Aging Cream* compared to untreated control on HDF cell line.

Table II: The quantitative expression level of the AQP3 gene in *Timeless Radiance-Anti-Aging Cream* and standard-treated cells (24 Hours).

Test item	Concentration (µg/mL)	Regulation in Terms of Folds
		AQP3
Cell Control	--	1.01 ± 0.16
Hyaluronic acid	250	1.95 ± 0.10
Timeless Radiance-Anti-Aging Cream	500	1.72 ± 0.15
	250	1.27 ± 0.15

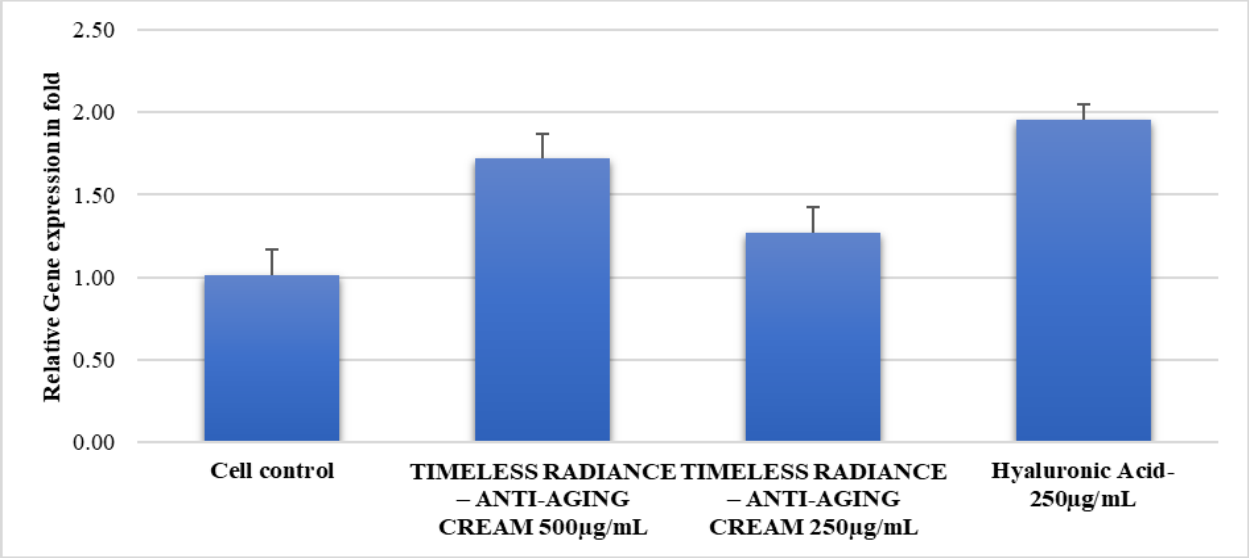

Fig. III: Relative gene expression of AQP3 in *Timeless Radiance-Anti-Aging Cream* and standard treated cells.

IV. DISCUSSION

Aquaporin-3 (AQP3) is predominantly expressed in the cell membranes of keratinocytes in the epidermis and is classified as an aquaglyceroporin, capable of transporting both water and glycerol. Among the thirteen aquaporins identified in humans (AQP0–AQP12), AQP3 is the most abundant in the skin and plays a pivotal role in maintaining hydration and barrier function. Its unique ability to facilitate the movement of water, glycerol, and even hydrogen peroxide underscores its multifaceted contribution to skin physiology. By regulating epidermal water permeability, AQP3 ensures optimal hydration and elasticity, while glycerol transport contributes to energy metabolism and lipid biosynthesis. Reduced AQP3 expression has been correlated with aged and photo-damaged skin, suggesting its decline is a key factor in dryness, impaired repair, and accelerated visible aging. Enhancing AQP3 activity, therefore, represents a promising molecular strategy for antiaging therapies. [4]

Parallel to this, the use of botanicals and natural formulations to combat skin aging has gained momentum due to their safety, cultural acceptance, and mechanistic alignment with molecular targets such as AQP3. Polyphenols, the most studied class of plant-derived secondary metabolites, exhibit potent antioxidant, anti-inflammatory, and anti-senescent properties. Structurally diverse subclasses, including flavonoids, phenolic acids and lignans, and stilbenes, have been shown to counter oxidative stress, delay senescence, and improve extracellular matrix integrity. In vitro models of cellular senescence and stress-induced premature senescence (SIPS) consistently demonstrate that polyphenols can modulate signalling pathways to reduce ROS accumulation, inhibit pro-inflammatory mediators, and enhance cell survival, thereby mitigating age-associated dermal deterioration. [5]

Polyphenols, widely distributed in plant extracts, have been extensively studied for their antioxidant and anti-inflammatory properties. Their capacity to neutralize reactive oxygen species (ROS), inhibit matrix metalloproteinases (MMPs), and delay stress-induced premature senescence (SIPS) makes them especially relevant for skin aging. Experimental models show that polyphenols can modulate signalling pathways such as MAPK and NF-κB, which are also implicated in the regulation of aquaporins. These findings suggest a mechanistic basis for the observed protective effects of polyphenol-rich botanicals on epidermal hydration and barrier function. [6]

Conventional anti-aging therapies, such as retinoids, peptides, and hyaluronic acid fillers, primarily target extracellular matrix remodelling or superficial hydration. While clinically effective, these approaches can be associated with irritation, cost, or invasive application methods. In contrast, herbal and natural formulations provide a complementary route that not only addresses oxidative and inflammatory pathways but may also influence molecular targets such as

AQP3. This positions botanical formulations as safer, sustainable, and potentially multifunctional alternatives within the cosmeceutical landscape.

For topically applied herbal formulations to exert their benefits, bioactive compounds must permeate the stratum corneum and reach the viable epidermis, where the AQP3 gene is localized. Molecular size, lipophilicity, and formulation design critically influence this process. Natural oils and lipid carriers have shown promise in enhancing penetration and improving the delivery efficiency of polyphenols and other phytochemicals. [7]

In this context, the present study explored *Timeless Radiance-Anti-Aging Cream*, a polyherbal formulation (Greenspace) enriched with bioactive natural ingredients such as *Ganoderma lucidum (*Reishi mushroom) extract, *Boswellia serrata* (Frankincense) hydrosol, *Crocus sativus (*Saffron) oil, *Prunus dulcis* (Sweet Almond) oil and *Aloe vera extract*. Reishi mushroom is renowned for its immunomodulatory polysaccharides and antioxidant triterpenes that help combat oxidative stress and support dermal regeneration. *Frankincense hydrosol* offers anti-inflammatory and skin-rejuvenating effects. [8], [9] Saffron brightens skin and reduces hyperpigmentation by suppressing melanin production. Due to its anti-inflammatory and antibacterial properties, *Saffron* is soothing to acne-prone skin, reduces inflammation, and prevents breakouts. [10] Sweet almond oil provides essential fatty acids that strengthen barrier integrity and improve hydration. [11] *Aloe vera* deeply hydrates, soothes irritations like sunburn and eczema, and helps manage acne by fighting bacteria and reducing inflammation. It also accelerates wound healing, boosts collagen for anti-aging, and can reduce the appearance of dark spots and scars. *Aloe vera* works by increasing collagen and hyaluronic acid production, increasing skin elasticity, and reducing the appearance of fine lines and wrinkles. [12] Together, these ingredients are hypothesized to synergistically enhance AQP3 expression, restore epidermal hydration, and reduce the clinical hallmarks of aging such as wrinkles, fine lines, and loss of elasticity.

Cosmeceuticals, when rationally designed, can shift from being purely cosmetic to functionally therapeutic. By targeting both hydration and cellular senescence pathways, herbal creams such as *Timeless Radiance* may represent an evidence-based strategy to complement or even substitute conventional antiaging treatments. Future clinical studies and mechanistic investigations into the direct effects of botanicals on AQP3 expression will be essential to validate these observations and establish their translational relevance.

V. CONCLUSION

Results from invitro evaluation of anti-aging cream suggests improved skin hydration, enhanced texture, and a visible reduction in early signs of aging with regular use. This herbal formulation offers a safer, natural alternative to synthetic anti-aging products, with potential for wide application in the cosmeceutical industry.

Timeless radiance anti-aging cream at 500 µg/mL for 24 hours resulted in a 1.72 ± 0.15-fold increase in AQP3 gene expression compared to untreated control cells. Since AQP3 plays a crucial role in the transport of water and glycerol across epidermal layers, its upregulation indicates the ability of the formulation to enhance skin hydration, maintain hydrostatic pressure, and improve skin texture. [13] These findings provide molecular evidence supporting the moisturizing efficacy and potential anti-aging properties of the formulation.

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VII. CONFLICT OF INTEREST

No conflict of Interest.

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