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The Effect of Wheat Adventitious Root and Callus Extracts on Protecting Skin Fibroblast Cells against Ultraviolet B

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ABSTRACT

Purpose: Sunburn, edema, hyperplasia, skin photo-aging, and skin cancer have been extensively associated with ultraviolet (UV) exposure. The role of natural phytochemicals in mitigating these UV-induced conditions has garnered significant scientific interest. Wheat (Triticum aestivum) is a rich source of polyphenols and has been shown to possess anti-inflammatory, anti-aging, and antioxidant properties in numerous in vivo and in vitro studies. Methods: This study used MTT assay, cell cycle analysis, scratch assay, and western blotting to investigate the protective effects of wheat adventitious root (WR) and wheat callus water (WC) and ethanolic (ER, EC) extracts on human skin fibroblast (HFF-2) cells against UVB-induced damage. Results: Among the prepared water and hydroethanolic extracts, the water extracts were selected for their superior performance in protecting cells against UVB-induced damage, as evidenced by MTT assays, enhanced cell growth, and total phenol content. The optimal doses were determined to be 0.1 µg/ml of WC extract and 10 µg/ml of WR extract. The observed protective effects included decreased reactive oxygen species (ROS), increased S/G2 phase indicative of DNA repair, upregulated Sirt-1 expression level, inhibition of matrix metalloproteinase-1 (MMP-1), and enhanced migration of fibroblast cells. Among the groups investigated (WR, ER, EC, and control), WC demonstrated the most pronounced protective effects. Conclusion: The study concluded that WC could pave the way for developing novel anti-aging and sunscreen products.

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Introduction

The skin, as the largest organ in the human body, is a protective barrier against environmental damage and has significant aesthetic importance, which also reflects the aging process.[1] Skin aging is associated with many factors, including intrinsic aging, exposure to ultraviolet radiation (UVR), and chemical pollutants. Oxidative stress, caused by reactive oxygen species (ROS), is widely recognized as one of the primary factors contributing to both intrinsic and extrinsic skin aging.[2] ROS cause DNA damage and induce mutation and cell death. It also stimulates the inflammatory process, causing skin redness. Previous studies have shown that antioxidant compounds in plant extracts can protect skin cells from cell death.[3] Previously known plant extracts contain various phytochemicals with various biological activities, including antioxidants, such as rice (*Oryza sativa*)[4], jasmine (*Jasminum sambac*)[5] , and roselle (*Hibiscus Sabdariffa L.*)[6] This property serves as the primary reason for using these plants in medicine.[7] Wheat (*Triticum aestivum*), classified as a cereal crop, plays an important role in human nutrition. Wheat has been developed for the treatment of various diseases due to its therapeutic potential in the treatment of various diseases, including heart disease, diabetes, ischemia, and skin diseases, and due to its antioxidant, anti-aging, and anti-cancer properties.[8] Wheat plant extract has been reported to contain vitamins and bioactive compounds such as phenolic compounds (ferulic acid). Polyphenols are known to be effective anti-aging agents.[9, 10] Polyphenols are known to be effective anti-aging agents. Guillou et al. showed that wheat extract significantly increased skin hydration and improved related clinical symptoms in women with dry skin.[11] Another study by Boisnic et al. demonstrated the potential anti-aging properties of wheat extract.[12] The many beneficial effects of polyphenols on skin health and appearance include reducing oxidative stress, increasing collagen and elastin production, and promoting skin cell regeneration.[13] Significant financial investments in cosmetics and anti-aging treatments highlight the demand for effective solutions and have stimulated ongoing research into the prevention and treatment of skin aging.[2] Plant cell culture has become increasingly important in the production of phytochemicals for medical, cosmetic, and food applications. While extensive research has focused on the medicinal properties of plant cell cultures, recent studies have emphasized their potential in the cosmetic and food industries. Thus, it has been proven that plant stem cells can delay aging and enhance skin regeneration.[14] Today, callus and cell suspension culture techniques are used to produce valuable secondary metabolites that are used as nutrients and pharmaceuticals.[15] Callus cultures offer the advantage of producing large quantities of biologically active substances with high reproducibility, in a relatively short time, in a limited space, and at a lower cost, without culturing the whole plant or using environmental pollutants. Recently, several companies have commercialized these products.[16]

Given the potential of wheat to develop anti-skin aging products and the benefits of plant calluses, to the best of our knowledge, while several studies have investigated wheat-derived extracts (*e.g.*, grain, bran) for antioxidant and skin-related applications, no studies have specifically evaluated wheat callus or adventitious root extracts in a UVB-induced photoaging model using human dermal fibroblasts, this study investigates, for the first time, the protective effects of water and ethanolic extracts of wheat callus (WC and EC) on skin fibroblasts exposed to UVB radiation, comparing them with water and ethanolic extracts of wheat adventitious root (WR and ER). In this study, we hypothesized that a) wheat callus extracts would exhibit stronger cytoprotective and antioxidant effects than adventitious root extracts due to differences in metabolite composition; b) aqueous or ethanolic extracts would demonstrate different biological activities; c) the protective effects would involve modulation of oxidative stress and key aging-related pathways, including upregulation of Sirt-1 and inhibition of MMP-1.

Materials and Methods

Materials

The following materials were used in this study: Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), phosphoric acid (H_3PO_4), Tween 20, sulfuric acid (H_2SO_4), gallic acid, ascorbic acid, quercetin, aluminum chloride ($AlCl_3$), 2,2-diphenyl-1-picrylhydrazyl (DPPH), collagenase from *Clostridium histolyticum*, N-[3-(2-furyl) acryloyl]-leu-gly-Pro-Ala, ethanol, phenol, RPMI, RNase A, and MTT (3-(4,5-dimethylthiazol-2-yl)-2, and 5-diphenyl tetrazolium bromide, a yellow tetrazolium) were purchased from Sigma-Aldrich (St. Louis, MO). Fetal bovine serum (FBS), Coomassie Brilliant Blue, and DMSO were obtained from Thermo Fisher Scientific (Waltham, MA). PBS, Triton X-100, Trypsin/EDTA (Cambridge, UK), trypsin, PVDF membrane, penicillin, streptomycin, and Tris-HCl buffer were purchased from GeneDirex Inc. (Gongye Rd, Taiwan). Rabbit antibodies, Sirt-1 and MMP-1 antibodies, were provided by Santa Cruz Biotechnology (Santa Cruz, CA).

Method

This study was conducted in two phases: (i) a screening phase evaluating all four extracts (WC, WR, EC, ER) using MTT assay under different treatment timings, and (ii) a mechanistic phase focusing on the best-performing extracts. Extracts demonstrating statistically significant cytoprotection (≥20% increase in viability compared to UVB control, $p<0.05$) were selected for further analyses including ROS, cell cycle, migration, and protein expression.

The Callus Culture

The seeds of *Triticum aestivum* were obtained from the East Azerbaijan Research and Education Center for Agriculture and Natural Resources. Initially, the seeds were sterilized by immersion in 70% ethanol for 2 minutes, followed by treatment with 5% bleach for 15 minutes, and subsequently washed several times with sterile distilled water. The sterilized seeds were then cultured on agar-based Murashige and Skoog (MS) medium. The culture medium was supplemented with 1 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) to induce callus formation and adventitious root development. The cultured calli were kept in an incubator for 8 hours daily under appropriate light, humidity, and a temperature of 24°C.[17]

Sample Preparation

One gram of callus and root tissue (dried at 38°C) was soaked in 10 ml of distilled water and 80% ethanol for 24 h. The samples were then placed in an ice bath and lysed using a power probe sonicator for 10 min, consisting of 10 sonication cycles of 1 min followed by a 1 min rest period to cool the samples at a frequency of 70 kHz and a half cycle (Hilscher, Germany). The lysates were centrifuged at 12,000 × g for 20 min at 4°C. The resulting extracts were stored at −20°C until further use.[4]

Determination of Total Polyphenolic Contents

To measure the phenolic content of the extracts, 100 microliters of the extracts were dissolved in 2 mL of 2% sodium carbonate. After 2 minutes, 100 µl of 1 N Folin-Ciocalteu reagent was added. The mixture was incubated for 30 minutes, and the absorbance was measured at 760 nm using an Ultrospec 2000 UV/Visible

spectrophotometer (Pharmacia Biotech Instruments, Ltd., Cambridge, England).[18] Phenolic content was calculated as gallic acid equivalent (GAE) micrograms.[19]

Determination of Total Flavonoid Contents

To determine the flavonoid content, 100 µl of the extract was diluted to a volume of 0.5 mL. Then, 30 µl of 5% NaNO2 was added, and after mixing for 5 minutes, 300 µl of 10% AlCl3 was added. After another 5 minutes, 200 µl of 1 N NaOH was added, and the absorbance was measured at 510 nm. A standard curve was prepared using quercetin (0-100 nmol), and the flavonoid content was reported as the equivalent of quercetin (QE) micrograms.[19]

Determination of Antioxidant Activity Using DPPH

The antioxidant activity of the callus and adventitious root extracts was examined using the DPPH method with slight modifications.[19] Briefly, 10 µl of callus extract (1mg/ml) was added to 90 µl of 0.2 mM DPPH solution. The mixtures were incubated for 30 minutes at 25°C in the dark, and the absorbance was measured at 517 nm. Radical scavenging activity was calculated using the equation:

% Inhibition = [(A0-A1)/A0] × 100

Determination of Total Carbohydrates

First, 500 µl of the extracts were mixed with 1 ml of 2% phenol. Then, 2.5 ml of 98% sulfuric acid was added dropwise to the solution. After 10 minutes, the solutions were cooled in cold water for 30 minutes, and their absorbance was measured at 490 nm.[20] A standard curve was prepared using glucose, fructose, and galactose (25, 50, 100, 150 µg/ml) at 490 nm (y = 0.0088x + 0.2376).

Solid Residue

First, a pre-weighed piece of aluminum foil was recorded. Subsequently, 100 µL of the sample was carefully dispensed onto the foil, which was then placed in an oven at 37 °C and dried for 24 hours. After drying, the foil was weighed again, and the dry weight of the sample was determined by subtracting the initial foil weight from the final measured weight.

Induction of Aging in Cells with UVB

Human fibroblast cells (HFF-2) were exposed to a dose of 60 mJ/cm UVB (λmax = 300 nm) for 30 s. The UV lamp was placed 20 cm from the dish. After UV exposure, the medium was replaced with fresh RPMI containing 10% FBS. (TL 40W-12 RS, Philips, The Netherlands). The formula used to determine the irradiation time was: UV energy (mJ/cm^2) = unit time (s) × UV intensity (mW/cm^2). Subsequently, the cells were treated with different doses of the extract and incubated at 37°C with 5% CO_2.[16, 21, 22] (Three treatment timings were used to distinguish mechanistic effects: (i) pre-treatment (24 h before UVB) to evaluate preventive/protective effects, (ii) immediate treatment to assess acute response, (iii) post-treatment (24 h after UVB) to evaluate repair/regenerative capacity.)

Proliferation Studies

MTT assay was used to evaluate the effect of the extract on the proliferation of Human Foreskin Fibroblasts (HFF-2) cells. A total of 10^4 cells per well of 96-well plates were cultured in quadruplicate in RPMI medium

containing 10% FBS and incubated at 37°C and 5% CO_2. After 24 hours, they were treated with different concentrations of callus and Adventitious Root extract (0.01, 0.1, 1, 10, 100 µg/ml). The treatment was performed in three stages: 24 hours before UVB, immediately after UVB, and 24 hours after UVB exposure. The duration of each treatment was 24 hours. The cells were incubated with MTT (5 mg/ml stock solution) for 3 hours at 37°C. Then, 200 µL of DMSO was added to each well and incubated for 30 minutes to dissolve the formazan crystals. Finally, an ELISA reader (STAT FAX 3200, Awareness Technologies, Minn) was used to read the absorbance of the formazan crystals at a wavelength of 570 nm.[23]

Cell Cycle Distribution

To evaluate the effect of the extract on the HFF-2 cell cycle, 4×10^5 cells were seeded in a 6-well plate (RPMI medium containing 10% FBS) and treated with WC and WR extracts at the optimum dose (0.1 µg/ml for WC and 10 µg/ml for WR) under UVB exposure, followed by 24 hours of incubation. The cell suspension was then fixed with 70% cold ethanol at -20°C for 24 hours. After centrifugation and washing with PBS, the cells were resuspended in PBS containing 10-50 µg/ml RNase A for 30 minutes. The cells were then incubated at 37°C in the dark for 30 minutes with PBS containing 1 µl/ml DAPI and 1 µl/ml Triton X-100, followed by flow cytometry analysis (BD-FACSC-000, FACSCalibur, Min).[24]

Intracellular Reactive Oxygen Species (ROS) Assay

Intracellular ROS generation was measured via flow cytometry analysis using cell-permeable redox-sensitive dye 2-7-dichlorodihydrofluorescein diacetate (DCFH-DA). In short, cells were cultured in 6-well plates (RPMI medium containing 10% FBS) and treated with WC and WR extracts at the optimum dose (0.1 µg/ml for WC and 10 µg/ml for WR) under UVB exposure for 48 h. Afterward, the cells were treated with DCFH-DA (100 µM, Sigma Aldrich, IL) and incubated for 40 min at 37°C. About 10^6 cells were harvested with trypsin-EDTA and centrifuged at 1300 rpm for 10 min. The cells were resuspended in PBS and washed twice. Then, the generation of ROS was measured using flow cytometry analysis, using 10,000 events per sample.[25]

DAPI staining

To examine the nuclear morphology of the HFF-2 cells, A 6-well plate was used to seed a density of 10^6 cells/well (RPMI medium containing 10% FBS) and incubated for 48h with WC and WR extracts at the optimum dose (0.1 µg/ml for WC and 10 µg/ml for WR) under UVB exposure for 48 h. The cell suspension was washed with 1× PBS and fixed with 4% paraformaldehyde for 10 min. Then, the stained cells with DAPI (1 mg/ml) were observed using a fluorescent microscope (Leica, Wetzlar, Germany).[25]

Scratch Assay

First, HFF-2 cells were cultured in a 12-well plate, and immediately after 24 hours of treatment with WC and WR extracts at the optimum dose (0.1 µg/ml for WC and 10 µg/ml for WR) and UVB exposure, a scratch was made on the cells using a sterile 200 µL pipette tip. Then, the cells were placed in a complete growth medium (RPMI-FBS10%) and photographed under a microscope at different times. The number of cells between the lines drawn at 0, 24, and 48 hours was counted. Cell migration was quantified by counting cells within the wound area.[26]

Western Blotting

HFF-2 cells were cultured at a density of 4×10^5 in 6-well plates and incubated for 24 hours. After WC and WR extracts at the optimum dose (0.1 µg/ml for WC and 10 µg/ml for WR) and UVB exposure, the cells were washed twice with cold PBS and lysed in lysis buffer (4°C) for 45 minutes. They were centrifuged at 25,000 rpm for 25 minutes, and protein estimation was performed using the Bradford method. The proteins were separated on a 12% SDS gel and transferred to a PVDF membrane. The membrane was blocked with 5% skim milk in 1× TBS-T (Tris saline buffer containing 0.05% Tween 20) at 4°C and immunoblotted with primary antibodies against MMP-1 and Sirt-1. After incubation with secondary rabbit antibodies (1:1000) for 1 hour, the signals were detected using an ECL reagent. The β-actin antibodies were used as the loading control for MMP-1 and Sirt-1. Band intensities were quantified using ImageJ software and normalized to β-actin. Data represent mean ± SD of three independent experiments.[26]

Statistical Analysis

All data results were analysed using GraphPad Prism (version 8.0). Statistical analysis was performed using One-Way ANOVA Post Hoc Tests (Tukey and Duncan). Where **** means $p \leq 0.0001$, *** means $p \leq 0.001$, and * means $p \leq 0.05$ was considered statistically significant. The data were analysed to determine the difference in means between groups.

Results and Discussion

This study demonstrates, for the first time, the protective and anti-aging properties of water and ethanolic extracts derived from wheat (*Triticum aestivum*) callus and adventitious roots against UVB-induced damage in human skin fibroblasts. Collectively, our findings show that WC and WR extracts, particularly the aqueous forms, exert significant antioxidant, cytoprotective, and pro-regenerative effects, supporting their potential use as active ingredients in topical skincare and anti-photoaging formulations.

Total Extract Characterization

Determination of Total Polyphenolic and Flavonoid Contents

Ethanol and aqueous extracts of adventitious roots and callus wheat did not show significant differences in total phenol (Table 1). The total flavonoid content in wheat callus and adventitious root extracts was quantified as follows: WC (2.21 ± 0.30 µg/mg), WR (0.48 ± 0.04 µg/mg), EC (4.71 ± 0.12 µg/mg), ER (4.90 ±0.73 µg/mg) (Table 1). Ethanol extracts showed higher flavonoid content, with ER demonstrating the highest and WR the lowest among the groups. Callus extracts exhibited superior protective effects, potentially due to their higher phenolic and flavonoid content, as shown in Table 1. Graphical analyses (Graph B) indicated that the compounds present in the extracts are more suitable for clinical use at concentrations below 0.01, suggesting a richer potency in calluses. In the study by Wu et al., *Phyllanthus emblica* was investigated. In comparison with this study, the extracted polyphenol from callus and adventitious root of wheat was more than that of *Phyllanthus emblica*.[27]

Determination of Antioxidant Activity Using DPPH

The reduction of free radicals by DPPH was measured at 517 nm using the extracts. The antioxidant activity varied among the extracts (Table 1), attributed to their phenolic contents and known sources of antioxidants. Significant

differences in antioxidant activity were observed between EC and both WC and WR, as well as ER and WC and WR ($p \leq 0.01$). However, no significant difference was observed between EC and ER ($P = 0.979$) or between WC and WR ($P = 0.526$). Subsequent assessment of ROS levels in control and treated cells showed that WC and WR extracts inhibited the ROS increase induced by UVB radiation, confirming their antioxidant properties as depicted in Table 1. Wheat extracts are recognized for containing several B vitamins, such as B1, B2, niacin (B3), B5, pyridoxine (B6), biotin (B7), and B9, contributing to their reported skin-care properties.[28] Recent emphasis on using wheat plants in anti-aging skin products underscores the presence of specific herbal ingredients.[29] In the study by Kandasamy et al., the antioxidant activity of *Centella Asiatica* was investigated. In comparison, the DPPH inhibition rate was higher in ethanolic extracts of wheat callus and adventitious roots and lower in aqueous extracts of *Centella Asiatica.*[30]

Determination of Total Carbohydrates

Analysis of carbohydrate content in the extracts revealed that ER and WC extracts had the highest (21.43 ± 4.12 µg/ml) and lowest (1.40 ± 0.16 µg/ml) amounts, respectively, compared to other extracts (Table 1). ER and WC caused a significant increase in total carbohydrates compared to other treatments. The extracts were found to contain high levels of carbohydrates (Table 1). Carbohydrates exhibit a humectant effect by retaining water in the skin through hydrogen bonding. However, their poor skin absorption can lead to ROS production when exposed to UV, exacerbating cellular damage. Pre-treatment with extracts 24 hours before UV exposure demonstrated a protective effect against UVB radiation, particularly notable in WC at lower concentrations and WR at higher concentrations compared to the control group. Post-UV exposure at 24 hours revealed a therapeutic effect, especially at lower concentrations of both callus extracts, whether water or ethanol. Compared to the study by Ahmad et al., who examined the callus extract of *Plantago Lanceolata* L.,[31] and Arezoumand et al., which investigated *Hurdeum vulgare* callus extract, wheat callus extract has a lower carbohydrate content.[26]

Solid Residue

A significant increase in solid residue was observed in alcoholic extracts compared to aqueous extracts. The highest value of DPPH was found in EC, while the lowest was in WR. The highest amount of flavonoids was related to ethanolic extracts (EC and ER). Also, ER had the highest total carbohydrate, while WR had the lowest. Finally, the highest solid residue was obtained for EC. All four extracts of WC, WR, EC, and ER were phytochemically analysed, but only two aqueous extracts were selected to continue the experiments from the cell cycle onwards.

In the initial phytochemical characterization, the ethanolic extracts contained higher levels of flavonoids and carbohydrates, consistent with previous reports that ethanol facilitates the extraction of polyphenolic compounds and polar lipids from wheat tissues. However, despite their comparatively lower flavonoid content, the aqueous extracts, especially WC, exhibited superior biological activity in nearly all cellular assays. This suggests that the water extracts may contain additional hydrophilic metabolites or synergistic phytocomplexes that are more readily bioavailable to fibroblasts. Such observations align with emerging evidence that plant cell culture-derived extracts frequently harbor unique metabolic signatures not directly reflective of their flavonoid content alone.[32]

Table 1. The total content of polyphenolics and flavonoids in the extracts of Wheat *Triticum aestivum* callus and adventitious Root. (water-adventitious Root extract = WR, Water-Callus extract = WC, hydroethanolic–adventitious Root extract=ER, hydroethanolic-Callus extract=EC)

Extracts	DPPH (%)	Flavonoid (µg QE mg−1)	Total phenol (µg GAE mg−1)	Total Carbohydrate (µg/g)	Solid residue (mg/g)
WR	4.60 ± 0.32^{b}	0.48 ± 0.02^{c}	18.60 ± 1.2^{a}	1.40 ± 0.02^{d}	20 ± 1.2^{c}
WC	5.10 ± 0.21^{b}	2.21 ± 0.3^{b}	21.06 ± 1.6^{a}	17 ± 0.92^{b}	13 ± 0.9^{d}
ER	29.25 ± 2.4^{a}	4.90 ± 0.2^{a}	18.02 ± 1.4^{a}	21.43 ± 1.5^{a}	24 ± 1.1^{b}
EC	31.04 ± 2.3^{a}	4.71 ± 0.3^{a}	17.59 ± 1.3^{a}	6.20 ± 0.4^{c}	27 ± 1.3^{a}

Values with different superscript letters (a, b, c, d) indicate statistically significant differences ($p < 0.05$) using Duncan's test, while identical letters indicate no significant difference. GAE: gallic acid equivalent, QE: quercetin equivalent.

Cellular Studies

MTT Assay

The proliferative effect of WC, WR, EC and ER extracts at concentrations of 0.01, 0.1, 1, 10 and 100 µg/ml was evaluated in triplicate on HFF-2 cells, 24 h after UVB exposure (Fig. 1A), 24 h before UVB exposure (Fig. 1B) and immediately after UVB exposure (Fig. 1C). WR treatment 24 h before UVB showed the highest growth rate of HFF-2 cells, which was significantly higher than the control group. Cell growth increased with increasing WR concentration. Also, WC extract supported the growth of HFF-2 cells before UVB exposure. The effective doses to prevent cell damage were identified as 0.1 µg/ml for WC and 10 µg/ml for WR, which significantly increased growth compared to the control ($p<0.05$) (Fig. 1). The aqueous extracts showed better activity than the ethanolic extracts in the MTT assay, with the aqueous callus extract performing better than the aqueous root extract ($p<0.05$). No cell growth was observed in cells exposed to UVB and immediately treated with the extracts (Fig. 1C), and a significant reduction in cell population was observed compared to the control ($p<0.05$). However, 24 h earlier (Panel B), most groups showed more than 100% viability compared to the control group, especially WC at lower concentrations and WR at higher concentrations (40-50% growth compared to the control group), indicating the efficacy of the aqueous extracts. After 24 h, the callus extracts at lower concentrations were shown to effectively increase cell growth (Panel C). Lower concentrations resulted in a 20% increase in cell growth compared to the control, with no significant difference at the 0.1 concentration compared to the control group. Higher concentrations showed lower growth rates than lower doses, indicating potential toxicity in these extracts. Doses of 0.1 µg/mL for WC and 10 µg/mL for WR were selected as effective doses for further studies (Fig 1). The dose of 100 µg/mL in WR was not significant despite higher cell growth than 10 µg/mL, so a lower dose with better performance was selected. The MTT assay results obtained in the present study indicate that WC and ectopic WR extracts have significant cytoprotective effects on human fibroblasts exposed to UVB, especially when administered 24 h before irradiation. The observed viability patterns, i.e., an increase above 100% at low WC concentrations (0.1 µg/mL) and protective effects of WR at higher concentrations (10 µg/mL), are consistent with

previously reported anti-aging and antioxidant activities of plant callus extracts. Dose-response analysis (0.01–100 μg/ml) revealed a non-linear relationship, with maximal efficacy at low concentrations for WC (0.1 μg/ml) and higher concentrations for WR (10 μg/ml). This 100-fold difference suggests distinct bioactive profiles and higher potency of callus-derived metabolites. The inverted U-shaped response observed for WC may reflect hormetic effects commonly reported for plant-derived bioactives.These concentrations are within ranges reported for highly potent phytochemicals, though translation to topical formulations requires further investigation.

A directly comparable study is the work of Buranasudja et al. on *Centella asiatica* callus extract, which used human dermal fibroblasts (BJ cells) and evaluated cell viability using the MTT assay under oxidative stress conditions (H_2O_2).[3] Similar to our findings, they reported that pre-treatment with callus extract significantly prevented H_2O_2-induced cytotoxicity and restored fibroblast viability toward basal levels. Their study demonstrated that low concentrations of callus extract were sufficient to exert strong cytoprotective effects, suggesting the presence of potent hydrophilic metabolites. This pattern parallels our findings, where WC displayed maximal protective efficiency at very low doses (0.1 μg/ml), indicating that callus-derived extracts may contain highly bioactive compounds not directly correlated with total polyphenol content alone.[3]

However, several notable differences exist between the two studies. While *Centella asiatica* callus extract primarily attenuated oxidative injury induced by chemical stress (H_2O_2), the present study utilized a UVB-induced photo-aging model, which more closely reflects extrinsic skin aging mechanisms such as ROS generation, DNA fragmentation, and apoptosis. Despite this difference in stressor, both studies aligned in showing that callus extracts maintain mitochondrial metabolic activity, indicating preserved cellular function following stress exposure. The protective effect of wheat callus extract appears particularly prominent when administered before UVB irradiation, suggesting that WC may activate pre-conditioning or antioxidant pathways that become active during subsequent UV exposure.

Another distinction lies in the dose response behavior. In the *C. asiatica* study, cytoprotective effects increased steadily with extract concentration, whereas our findings suggest a non-linear relationship, where low doses were more effective than higher doses that tended to reduce viability, likely due to phytochemical overload or mild cytotoxicity at high concentrations. This inverted-U pattern is frequently observed in plant-derived extracts and underscores the superior potency of WC at sub-microgram concentrations.

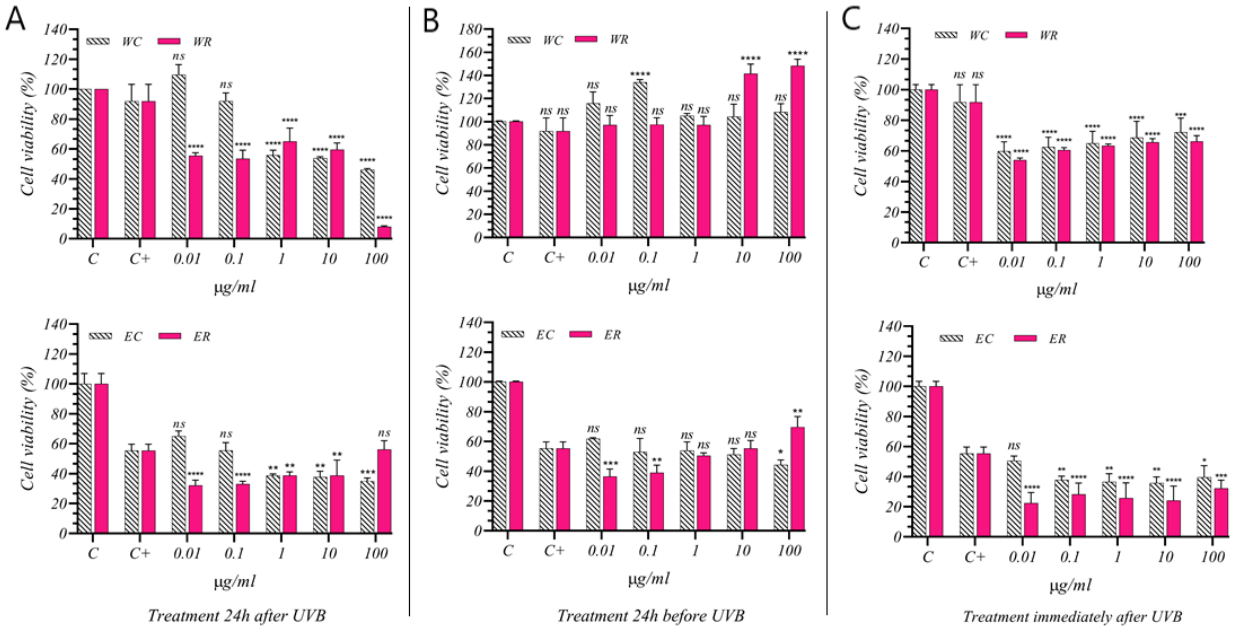


Fig 1. MTT assay showing the effect of WC and WR extracts on HFF-2 cell proliferation following UVB exposure (n=3). The MTT analysis of normal cells after 48 h incubation with different concentrations of extract was measured, treated 24 h after UVB (A), treated 24 h before UVB (B), and immediately treated after UVB (C). Statistical analysis was performed using One-Way ANOVA Post Hoc Tests (Tukey) ($p<0.05$). (****P≤0.0001, ***P≤0. 001 and **P≤0. 01). Control (C) and Positive Control (C+). Water extract of callus = WC, Water adventitious root = WR, Ethanol extract of callus = EC and Ethanol adventitious root = ER. Human Foreskin Fibroblasts (HFF-2)

Cell Cycle

Analysis of the extract's effect on the cell cycle of HFF-2 cells revealed that UVB exposure caused 38.8% of control cells to enter the sub-G1 phase, indicative of cell death initiation, and significantly reduced the number of cells in the G2 phase. Treatment with WC and WR extracts effectively inhibited cell progression to the sub-G1 phase and supported cell cycle progression. Consequently, 43.5% of cells treated with WC and 42.6% with WR extracts were in the G2 phase, a significant difference from the control group ($p>0.05$) (Fig 2). The effects of WC and WR extracts on the cell cycle were also investigated. UVB radiation induces cells to accumulate in the sub-G1 phase, indicating DNA damage and apoptosis. Conversely, cells treated with WC and WR extracts showed a higher concentration in the G2 phase, a period of intense protein synthesis and preparation for mitosis. Additionally, the number of cells in the S phase, responsible for DNA synthesis and replication during interphase before mitosis or meiosis, significantly increased compared to control cells.[33] The cell cycle analysis revealed that UVB exposure drove fibroblasts into the sub-G1 phase, indicative of DNA fragmentation and apoptosis. Treatment with both aqueous extracts significantly prevented this transition and facilitated redistribution of cells into the S/G2 phases, reflecting active DNA repair and preparation for mitosis. An increase in S/G2 phase was observed, consistent with activation of cell cycle checkpoints following UVB-induced stress. While this may reflect DNA repair processes, it could also indicate transient cell cycle arrest; therefore, this finding should be interpreted cautiously.These data, in conjunction with the DAPI staining results, which showed preserved nuclear morphology, reinforce the capacity of wheat-derived extracts to mitigate genotoxic stress. This outcome is consistent with other plant-derived antioxidants, such as those from pomegranate and Rhaponticum species, which have been shown to sustain DNA integrity and promote repair under oxidative stress.[33,34,36] Several independent studies of plant callus or phytochemical extracts have reported protection of dermal cells against UV or oxidative damage that manifests as preserved proliferation and altered cell-cycle distributions. For example, callus extracts

from *Centella asiatica* and other plant calluses protected fibroblasts from oxidative/UV damage and preserved proliferative capacity in vitro, reporting similar endpoints (cell viability, reduced markers of DNA damage, and protection of cell-cycle progression). These findings echo our observation that aqueous wheat callus extract preserves S/G2 populations after UVB exposure.[3]

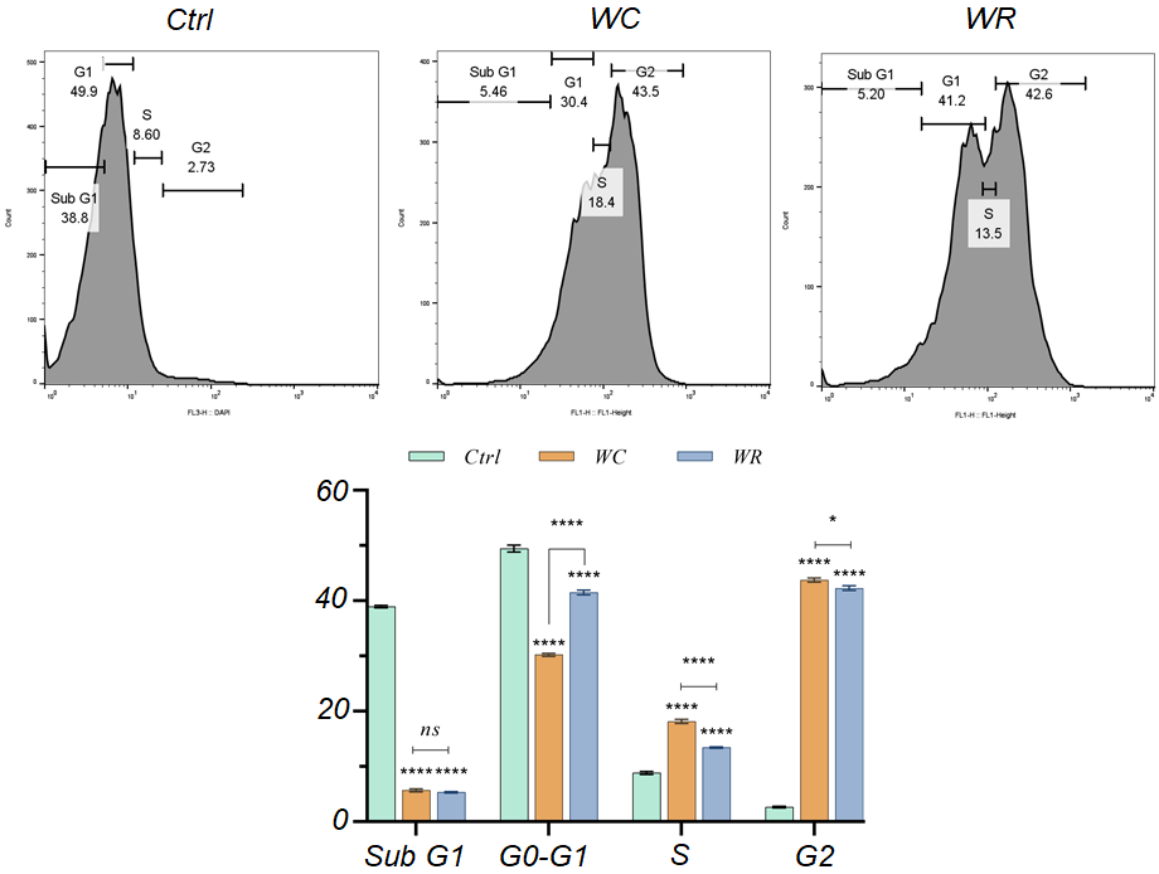


Fig 2. Cell cycle measurement of the extract's optimum dose effect on different cell cycle phases after 48 h (n=3). Statistical analysis was performed using One-Way ANOVA Post Hoc Tests (Tukey) ($p<0.05$). (****P≤0.0001, ***P≤0. 001 and **P≤0. 01). Water extract of callus = WC, Water extract of adventitious root = WR. Human Foreskin Fibroblasts (HFF-2).

Intracellular Reactive Oxygen Species (ROS) Assay

To evaluate the antioxidant effect of the extracts, intracellular ROS levels were measured in the cells. The ROS level in Unstain cells was 3.27%, but UVB exposure increased the ROS level in control cells by 82.8%. In contrast, treatment with WC and WR extracts significantly reduced the ROS level to 4.22% and 2.92%, respectively. ROS levels of WC and WR-treated cells were not significantly different from UVB-untreated control cells ($p > 0.05$). (Fig. 3). Phenolic compounds, which are widely distributed in plants, are known to be potent inhibitors of ROS. Both WC and WR extracts showed significant efficacy in reducing ROS levels (Fig. 3), highlighting their role as natural antioxidants capable of reducing oxidative DNA damage.[35] Importantly, ROS suppression occurred even at very low concentrations of the extracts, supporting the hypothesis that their protective capacity relies on highly potent antioxidant compounds or synergistic combinatorial interactions. In a study by Pacheco-Palencia et al., the protective effects of a standardized polyphenolic extract of pomegranate (*Punica granatum* L.) on human skin fibroblasts exposed to UV radiation were investigated, indicating preservation of the cell cycle and DNA synthesis.[36] Although no direct comparison with standard antioxidants (e.g., resveratrol, vitamin C) was

performed, the magnitude of ROS reduction observed is comparable to values reported for established phytochemicals in similar models.

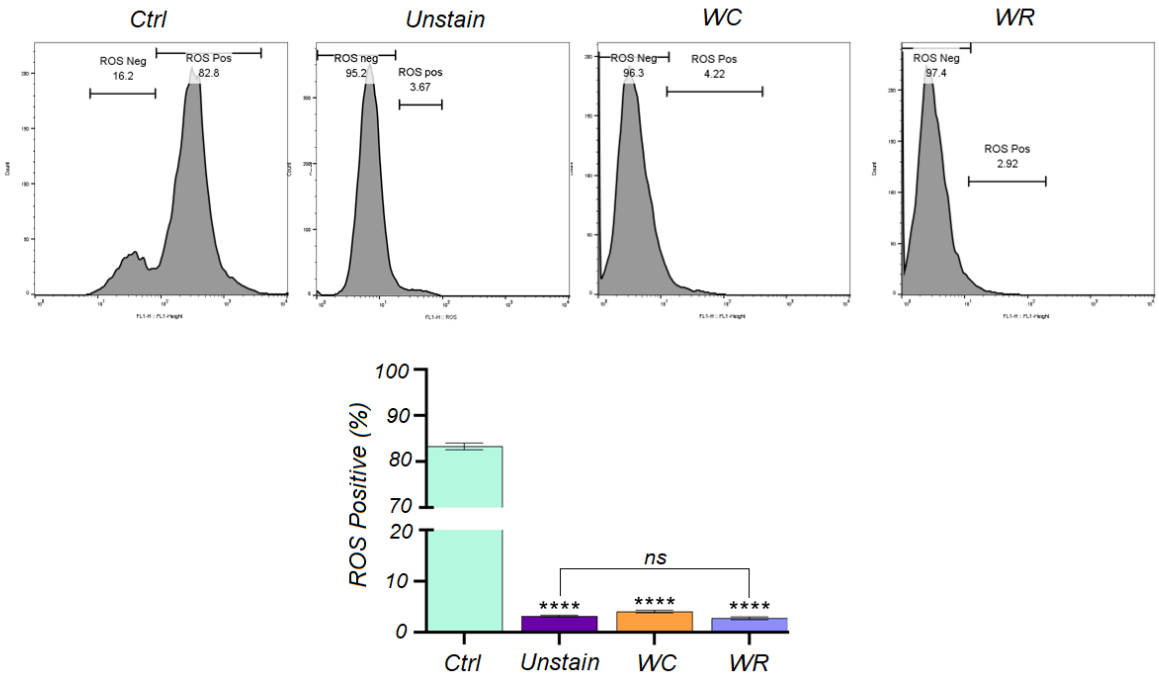


Fig 3. The effect of optimum extract dose on the amount of reactive oxygen species (ROS) was measured at the cellular level after 48 h (n=3). Statistical analysis was performed using One-Way ANOVA Post Hoc Tests (Tukey) ($p<0.05$). (****P≤0.0001, ***P≤0. 001 and **P≤0. 01). Water extract of callus = WC, Water extract of adventitious root = WR. Human Foreskin Fibroblasts (HFF-2).

DAPI Staining

DAPI staining was utilized to evaluate nuclear changes and proliferation in HFF-2 cells (Fig 4). Morphological examination and cell count indicated that WC and WR extracts maintained cell nuclear morphology and increased cell numbers compared to the untreated cells. Furthermore, DAPI fluorescent staining was employed to assess cellular morphology. The results depicted in Fig 4 reveal that the DNA in control cells was damaged, whereas cells treated with the extracts exhibited intact DNA. Additionally, the treated cells demonstrated a higher cell count than the control group. This contrasts with the study by Devi et al., which examined the impact of *Terminalia chebula* extracts on keratinocyte and fibroblast cell proliferation and produced superior findings.[37] The preservation of nuclear morphology observed after treatment is consistent with the extracts' antioxidant activity (DPPH and intracellular ROS data) and their ability to maintain cells in S/G2 phases—periods associated with active DNA replication and repair. Reduced ROS likely lowers oxidative DNA lesions and the subsequent activation of apoptotic cascades, while elevated Sirt-1 expression (observed by western blot) could contribute to chromatin stability and DNA repair signaling; SIRT1 has been implicated in promoting genome maintenance under stress. Thus, the morphological preservation seen with DAPI staining is plausibly linked to the antioxidant and SIRT1-modulating activities of the wheat extracts.[38]

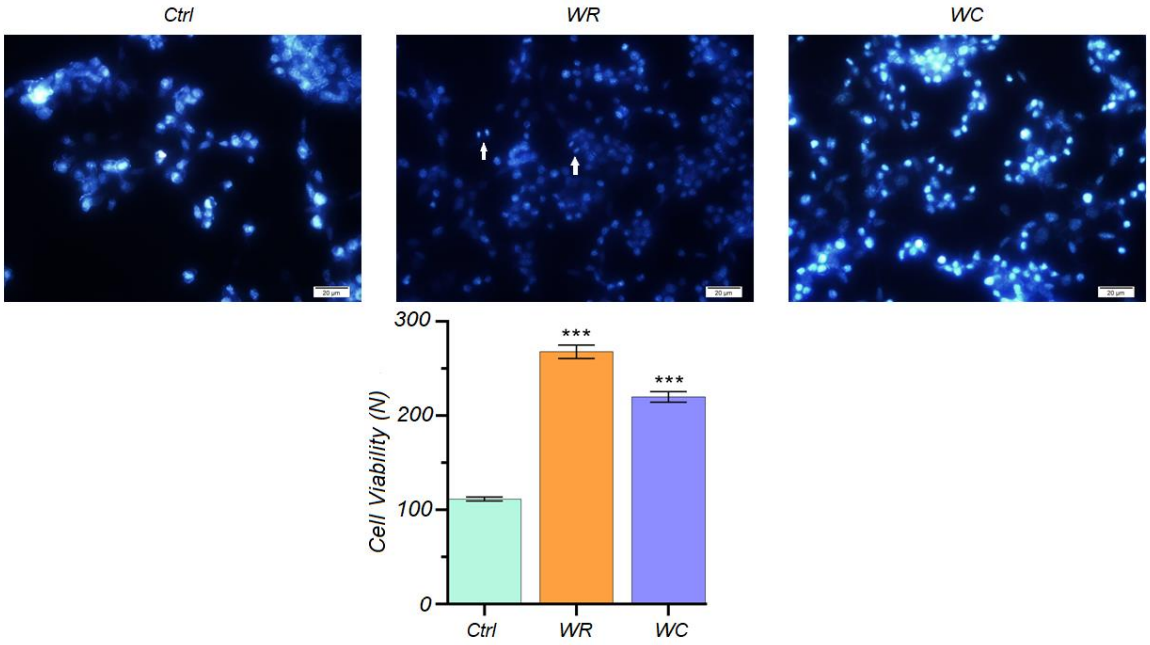


Fig 4. The representative nuclei stained with DAPI in HFF-2 cells were treated with the extract after 48 hours of incubation (n=3). Statistical analysis was performed using One-Way ANOVA Post Hoc Tests (Tukey) ($p<0.05$). (****P≤0.0001, ***P≤0. 001 and **P≤0. 01). Arrows indicate cell division. Water extract of callus = WC and adventitious root = WR. Human Foreskin Fibroblasts (HFF-2).

Wound Scratch Assay

The rate of HFF-2 cell migration in the presence of WC and WR extracts was assessed using a scratch assay (Fig 5). Images showed significant cell migration at 24 and 48 hours. WR-treated cells exhibited a higher migration rate (12.5%) compared to WC and control cells (exposed to UVB) (35%). The migration of HFF-2 cells in response to the extracts was evaluated using the scratch assay method. The number of cells within the scratched area was quantified after 24 and 48 hours until complete closure of the scratch by cell migration. Extract-treated wells exhibited a higher cell count than the control, indicating enhanced cell migration for wound repair. WR extract demonstrated the most effective performance in this assay, significantly promoting cell migration (Fig 5). The presence of phenolics and flavonoids in the extracts correlates with their potential for tissue repair. Previous studies have highlighted the role of phenols and flavonoids in stimulating fibroblast proliferation and facilitating the formation of new blood vessels and capillaries.[35, 39] Although WC exhibited superior antioxidant and anti-photoaging activity overall, WR demonstrated higher migration potential in the scratch assay. This suggests that the biological effects of wheat extracts are context-dependent, where WC predominantly modulates oxidative stress and aging-related pathways, whereas WR may contain bioactive compounds that preferentially enhance cytoskeletal reorganization and cell motility. These findings are consistent with those reported by Addis et al., underscoring the beneficial impact of plant extracts on fibroblast migration. Such enhancements in cell migration hold promise for accelerating wound healing processes.[40] A study by Buranasudja et al. investigated the skin anti-aging effects *of Centella Asiatica* callus extract. Results show that plant callus extracts increased the expression of antioxidant enzymes and the anti-skin-aging potential in human foreskin fibroblast cells [3]. The results of our study are in line with this study. Some studies show that plant callus extracts have multiple beneficial effects on the skin. Moon et al.,[41] obtained extracts using the Haman region lotus-derived callus, enabling a controlled,

quantitative, and infinite supply. In conclusion, they found that the lotus-derived callus extract has anti-inflammatory, antioxidant, and skin-soothing properties and plays an essential role in skin whitening. Therefore, they propose that the lotus-derived callus extract has the potential to become a new raw material for the cosmetic industry.

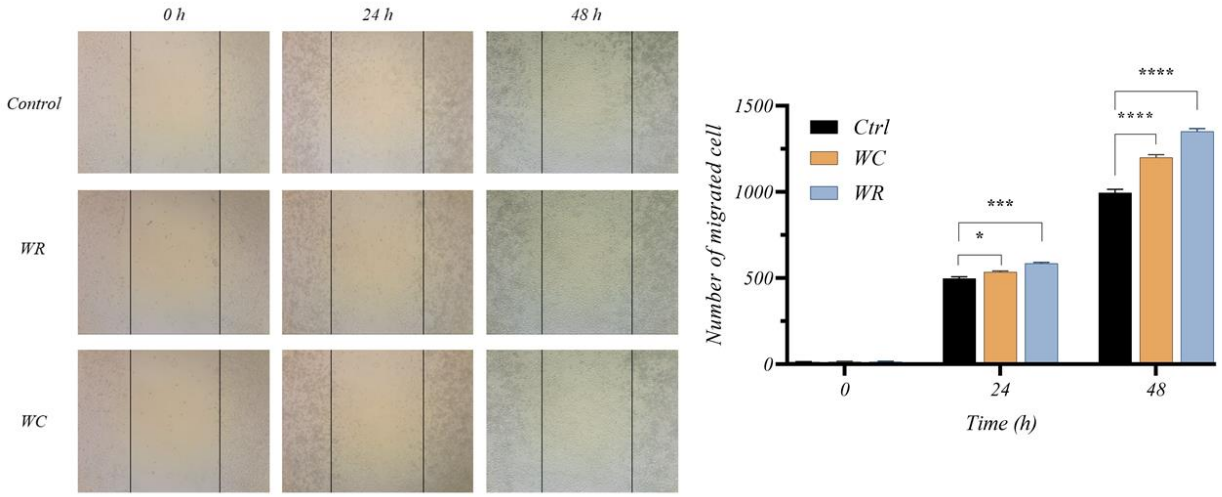


Fig 5. HFF-2 cells have migrated after scratch induction and treatment. All pictures were taken at times 0, 24, and 48 hours (n=3). The number of cells between the lines drawn at 0, 24, and 48 hours was counted. Images were taken at 50× magnification. Statistical analysis was performed using One-Way ANOVA Post Hoc Tests (Tukey) ($p<0.05$). (****P≤0.0001, ***P≤0. 001 and **P≤0. 01). Water extract of callus = WC, Water extract of adventitious root = WR. Human Foreskin Fibroblasts (HFF-2).

Western Blotting

Western blot analysis compared the expression of MMP-1 and Sirt-1 between extract-treated and untreated cells (Fig 6). WC extract demonstrated inhibition of MMP-1 and stronger expression of Sirt-1 compared to WR extract, highlighting the extracts' potential for anti-aging and antioxidant applications. The β-actin antibody was used as a loading control for MMP-1 and Sirt-1. Additionally, β-actin served as a loading control to normalize protein expression, while untreated HFF-2 cells acted as the negative control (Protein expression levels were normalized to β-actin and quantified using densitometric analysis (ImageJ). Data are presented as mean ± SD from three independent experiments), Representative blots correspond to each experimental condition, with β-actin shown for each lane as loading control.UVB irradiation of skin cells disrupts crucial signaling pathways involved in skin aging, prominently altering the expression of the MMP1 gene and decreasing SIRT1 expression (Fig 6). MMPs are a group of endopeptidases that degrade extracellular matrix proteins, with activities spanning synthesis (gene expression), activation, and proteolytic inhibition.[42] UVB radiation induces MMP gene expression, leading to the degradation of skin fibrillar collagen and the subsequent formation of wrinkles and sagging.[16] Treatment with WC and WR extracts significantly attenuated UVB-induced MMP1 expression ($p \leq 0.01$) and MMP1 induction triggered by UVB. Enhanced SIRT1 expression in skin cells mitigates signs of skin aging,[44] while reduced SIRT1 expression accelerates aging.[44] UVB exposure notably diminishes SIRT1 expression in skin fibroblasts. Conversely, treatment with WC and WR extracts appeared to dose-dependently increase SIRT1 expression in UVB-exposed cells (Fig 6). UV radiation exposure can also induce apoptosis in skin cells by activating caspases.[45] Son et al. investigated the inhibitory effect of *Prunus mume* seed on skin aging, focusing on SIRT1 and MMP-1. They found that *Prunus mume* significantly reduced MMP-1 compared to the UVB-irradiated group and increased SIRT1 protein level under treatment.[46] Our results are consistent with their findings, supporting the anti-aging potential of *Ocimume. In* the study by Manjudevi et al., the production and extraction of bioactive metabolites by

the callus of *Ocimum* were investigated. The results of RT-qPCR showed that cellular antioxidant enzymes present in callus extract reduce oxidative stress. In addition, callus extract inhibited the induction of MMP-9, indicating its potential antioxidant and antiaging activity.[47] Our western blot analysis showed that treatment with WC markedly attenuated UVB-induced MMP-1 expression while concomitantly increasing Sirt-1 protein levels compared with UVB-exposed controls and WR-treated cells. These changes provide a plausible molecular explanation for the phenotypic protection observed (reduced ROS, preserved nuclear morphology, improved cell viability, redistribution into S/G_2, and enhanced migration). MMP-1 is a principal collagenase responsible for the degradation of fibrillar collagens in dermal extracellular matrix; therefore, its suppression by WC is consistent with an anti-photoaging profile that would limit collagen breakdown and wrinkle formation. Conversely, upregulation of Sirt-1, a NAD^+-dependent deacetylase implicated in stress resistance, DNA repair, and metabolic regulation, likely contributes to enhanced cellular resilience: Sirt-1 activation has been shown to promote DNA repair pathways, suppress pro-inflammatory transcription factors (for example, NF-κB), and negatively regulate AP-1 signaling that drives MMP transcription.[48, 49] Together, reduced MMP-1 and increased Sirt-1 create a dual protective axis that both diminishes matrix degradation and enhances intrinsic repair and survival pathways in fibroblasts. How these protein changes relate to ROS, cell cycle, and migration. The observed decrease in intracellular ROS following WC/WR treatment provides an upstream explanation for altered MMP-1 and Sirt-1 expression. Oxidative stress caused by UVB activates MAPK cascades and AP-1, which in turn increase MMP-1 transcription; by scavenging ROS, wheat extracts likely blunt MAPK/AP-1 signaling and downstream MMP induction. Meanwhile, higher Sirt-1 expression can directly or indirectly favour cell-cycle checkpoints and DNA repair (consistent with the higher S/G_2 fraction we measured), because Sirt-1 deacetylates proteins such as p53 and components of the DNA-repair machinery, promoting controlled repair rather than apoptosis. Regarding migration, a tightly regulated decrease in MMP-1 can facilitate organized matrix remodelling required for directed cell movement; excessive MMP activity disrupts the ECM architecture and impairs effective migration, whereas the balanced modulation seen here supports scratch-closure and wound-healing behavior.[50, 51]

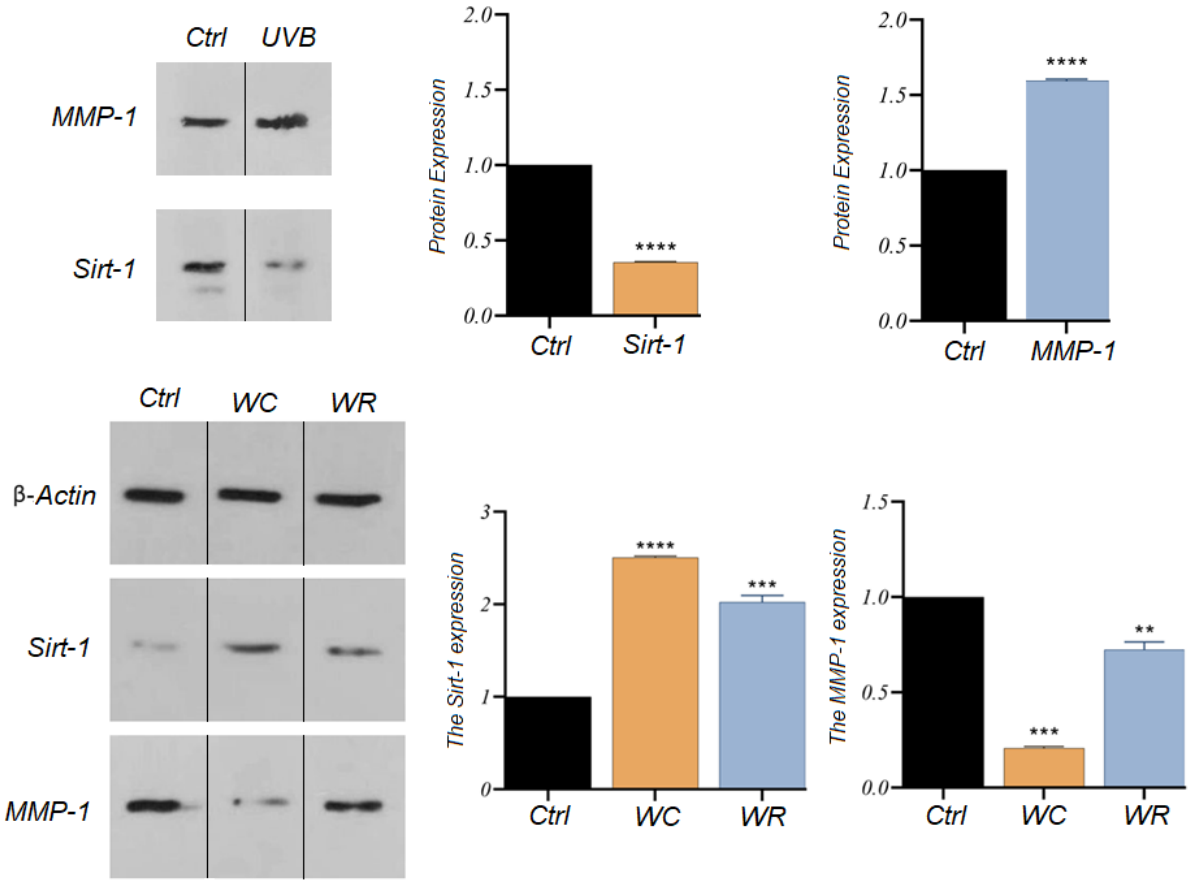


Fig 6. Comparison of the gelatinolytic activity on MMP-1 and Sirt-1 between the extracts after 48-hour incubation (n=3). Statistical analysis was performed using One-Way ANOVA Post Hoc Tests (Tukey) ($p<0.05$). Representative blots correspond to each experimental condition, with β-actin shown for each lane as loading control. (****P≤0.0001, ***P≤0. 001 and **P≤0. 01). Water extract of callus = WC, Water extract of adventitious root = WR.

Most published models in the field of plant-based anti-aging use "herbal" plants such as Centella asiatica (rich in triterpenoid saponins) or extracts derived from seeds/bran or stem cells of cereals such as *Oryza sativa.*[4] For example, studies using *C. asiatica* focus on its terpenoid fraction to stimulate collagen/fibronectin synthesis, wound healing, or suppress senescence under oxidative stress.[3, 30] In contrast, our manuscript explores water-soluble metabolites from wheat callus and expands the diversity of plant sources for anti-aging applications. This may reveal new bioactive molecules not covered by more common plants. Several previous works focus on stimulating extracellular matrix (ECM) synthesis, e.g., increasing collagen/fibronectin production in fibroblasts after treatment with *C. asiatica*. Other cereal-derived extracts (e.g., black rice or rice bran) focus primarily on antioxidant capacity, melanin inhibition, or tyrosinase/whitening effects for cosmetic benefits. In contrast, our data show that wheat callus extract modulates key proteins associated with aging and photoaging (increasing SIRT-1 and decreasing MMP-1), reduces ROS, influences cell cycle distribution, maintains viability, and improves fibroblast migration after UVB exposure. This is a broader and more integrated readout of skin cell health, combining antioxidant responses, protease regulation, cell survival, and repair/migration, and thus provides an arguably more comprehensive anti-aging model. Overall, the study identifies WC as the most promising candidate for further development. Its broad-spectrum activity, ranging from ROS reduction and DNA protection to stimulation of cell migration and regulation of aging-related proteins, positions it as a multifunctional ingredient with strong potential for integration into cosmeceutical products targeting skin rejuvenation and photoprotection.

Conclusion

This study provides the first comprehensive evidence that water-derived extracts from wheat (*Triticum aestivum*) callus and adventitious roots possess potent protective and anti-aging properties against UVB-induced damage in human skin fibroblasts. Among the four extracts evaluated, the WC consistently demonstrated the strongest bioactivity across all experimental endpoints. WC effectively reduced intracellular ROS levels, preserved nuclear integrity, restored normal cell-cycle distribution by promoting S/G2-phase DNA repair, enhanced fibroblast migration, and modulated two key molecular markers of photoaging, suppressing UVB-induced MMP-1 expression while upregulating the longevity-associated protein Sirt-1. The WR also exhibited significant cytoprotective and antioxidant effects, though to a lesser extent than WC.

water extracts exhibited stronger biological activity than ethanolic extracts despite lower flavonoid content. This suggests that hydrophilic compounds, such as peptides, polysaccharides, vitamins, or synergistic metabolite complexes, may play a dominant role in UVB protection. Moreover, antioxidant activity is not solely dependent on flavonoid concentration, but also on synergistic interactions between multiple phytochemicals. The ability of WC to act at very low concentrations further underscores its potency and suitability for cosmetic and dermatological applications. Collectively, these findings highlight wheat callus extract as a promising multifunctional ingredient capable of mitigating oxidative stress, preventing extracellular matrix degradation, and supporting cellular repair and regeneration following UVB exposure.

Future studies should investigate the specific active compounds responsible for these effects, evaluate extract performance in 3D skin models or *ex vivo* human skin, and explore formulation strategies for topical delivery. Overall, this work expands the current landscape of plant cell culture–derived cosmetic actives and identifies wheat callus extract as a strong candidate for the development of next-generation anti-aging and photoprotective skincare products. On the other hand, the promising results of this study suggest that callus extracts from other wheat varieties should be further evaluated for skin rejuvenation potential.

Limitations of the study

No topical anti-aging products derived from wheat or any other plant callus were found to compare with the positive control product in this study. Furthermore, applying the anti-aging creams to the cells caused unacceptable results, mainly because of the cell growth media color change and precipitation of cream ingredients in the medium. While no wheat-based anti-aging product was available for comparison, future studies could use a standard antioxidant, such as ascorbic acid, as a positive control. This study is limited by its in vitro design using a single cell type (HFF-2) and does not account for complex skin architecture. UV-A and UV-C were not evaluated. No in vivo validation or skin penetration studies were conducted. The specific active compounds responsible for the observed effects remain unidentified. Additionally, scalability and standardization of wheat callus production require further investigation.

Ethical Approval

This study did not involve human participants or live animals. All experiments were performed using commercially available cell lines and therefore did not require ethical approval.

Author contributions

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Formal analysis: Hamed Hamishehkar
Investigation: Azam Salmasi
Methodology: Morteza Kosarinasab
Project administration: Hamed Hamishehkar
Resources: Azam Salmasi
Software: Sajjad Sedaghat and Parviz Abroumand Azar
Supervision: Hamed Hamishehkar
Validation: Hamed Hamishehkar and Parviz Abroumand Azar
Writing–original draft: Azam Salmasi
Writing–review & editing: Hamed Hamishehkar

Data availability

All data generated or analyzed during this study are included in this published article.

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