The Effect of Propolis Extracts on Healthy Vascular Aging Mechanism by The Telomere Regulation in HUVEC Cells

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ABSTRACT

Introduction: Propolis is a very important natural bioactive product with potent antioxidant properties; however, the effects of Kazakhstan propolis on telomere-associated molecular pathways remain largely unexplored. The objective was to comparatively evaluate the antioxidant activity, total phenolic and flavonoid contents, cytotoxicity, and telomere-related gene expression profiles of different solvent extracts obtained from propolis collected from four regions of southern Kazakhstan.

Methods: Ethanolic, methanolic, water, and ethyl acetate extracts of four propolis samples (Pro1–Pro4) were prepared. Total phenolic content, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, total flavonoid content, and HUVEC cell viability were determined. Relative expression levels of *TERT*, *TERF1*, *TERF2*, and *PINX1* were quantified by qRT-PCR using the fold change ΔΔCt method with *GAPDH*.

Results: Methanolic and ethanolic extracts generally showed the highest phenolic and flavonoid contents and antioxidant activity. All extracts showed low cytotoxicity toward HUVEC cells, indicating good cellular compatibility. Gene expression analysis demonstrated that methanol extracts increased *TERF1* and *TERT* expression, particularly in the Pro2 and Pro3 samples, whereas ethanol extracts produced intermediate responses, and aqueous extracts showed comparatively weaker effects. *TERF2* showed a significant overall group effect, without significant pairwise differences, while *PINX1* showed variable but non-significant alterations among treatment groups. One-way ANOVA revealed significant differences for *TERF1*, *TERT*, and *TERF2*.

Conclusions: These data suggest that phenolic-rich propolis extracts may support endothelial health and are associated with altered expression of telomere-related genes, and highlight Kazakhstan propolis as a promising source of bioactive compounds for further vascular-aging research.

Keywords: Anti-aging, Antioxidant activity, Endothelial cells, HUVEC, Kazakhstan propolis, qRT-PCR, Telomere

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1. Introduction

The global increase in life expectancy has been accompanied by a rise in age-related disorders, making healthy aging a major biomedical challenge. Among the mechanisms underlying aging, oxidative stress is a principal driver of endothelial dysfunction, chronic inflammation, genomic instability, and telomere shortening. Excess reactive oxygen species disrupt redox homeostasis and damage proteins, lipids, and nucleic acids. Because vascular endothelial cells are continuously exposed to circulating oxidants and inflammatory mediators, they are particularly susceptible to oxidative injury. Consequently, preservation of endothelial integrity is an important target for preventing cardiovascular and age-associated diseases [1].

Telomeres are nucleoprotein structures at chromosome ends that preserve genomic stability during cell division. Progressive telomere shortening is a hallmark of cellular aging and is strongly influenced by oxidative stress. Oxidative lesions within telomeric regions are repaired less efficiently, accelerating telomere attrition and premature senescence. Telomere integrity depends on coordinated regulation of telomeric repeat-binding factor 1 (*TERF1*), telomeric repeat-binding factor 2 (*TERF2*), telomerase reverse transcriptase (*TERT*), and PIN2/TERF1-interacting telomerase inhibitor 1 (*PINX1*). These genes may be associated with endothelial dysfunction, impaired tissue regeneration, and chronic diseases. Thus, strategies that reduce oxidative stress while maintaining telomere homeostasis have attracted increasing attention as potential interventions against cellular aging. Telomere shortening triggers apoptosis or senescence [2]. Telomerase is the principal enzyme responsible for maintaining telomere length [3] and is a ribonucleoprotein complex composed of the telomerase RNA component (TERC) and catalytic reverse transcriptase subunit (*TERT*) [4]. Although telomerase activity is minimal in most normal somatic cells, it is highly active in stem and cancer cells, highlighting its role in cellular aging and proliferation [4,5].

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Natural products are rich sources of bioactive molecules targeting oxidative stress-related pathways. Propolis has gained considerable attention because of its diverse phytochemical composition and broad biological activities. Produced by honey bees from plant resins mixed with beeswax and salivary enzymes, propolis contains more than 500 identified compounds [6], contributing to its cardioprotective, anti-inflammatory, antioxidant, antimicrobial, immunomodulatory, antiviral, and anticancer properties. Many of these effects involve oxidative stress-sensitive pathways, including Nrf2, NF-κB, and MAPK [1,7]. Its protective effects on vascular endothelium are particularly noteworthy. Propolis has been shown to reduce intracellular ROS and lipid peroxidation, preserve nitric oxide bioavailability, inhibit endothelial apoptosis, and attenuate inflammatory signaling, suggesting effects on both oxidative balance and cellular longevity [8,9].

Propolis activity varies considerably with geographical origin, local flora, climatic conditions, bee species, harvesting season, and extraction methodology. Different solvents influence the phenolic contents, resulting in distinct phytochemical profiles and antioxidant and cytoprotective potentials. Comparative evaluation of solvent-dependent extracts is therefore essential for identifying biologically active preparations and understanding their mechanisms. Most previous studies have focused on antioxidant or antimicrobial activity, whereas less attention has been given to endothelial biology and telomere-associated responses. Thus, the potential effects of Kazakhstan propolis on telomere regulatory pathways remain largely unknown.

HUVECs are widely used to investigate endothelial physiology, oxidative stress, vascular aging, and cytoprotective mechanisms. Because endothelial dysfunction is an early event in numerous chronic diseases, HUVECs provide an appropriate model for evaluating natural compounds. Simultaneous assessment of antioxidant activity, cytotoxicity, cellular morphology, and telomere-associated gene expression provides a more comprehensive

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evaluation of endothelial protection than antioxidant assays alone. Endothelial aging is a major risk factor for cardiovascular disease, while telomere shortening is a key biomarker of this process [10]. Therefore, HUVECs provide a suitable in vitro system for investigating telomere-related responses.

Accordingly, this study comparatively investigated propolis samples collected from four geographically distinct regions of southern Kazakhstan. This study aims to present new data on the effects of Kazakhstan propolis extracts on the expression of telomere-associated genes in HUVECs, and to explore their possible relevance to telomere regulation in vascular aging.

2. Materials & Methods

2.1. Propolis Samples and Extraction

Samples were obtained from four geographically distinct locations in southern Kazakhstan (Table 1). Then, 5 g of each sample was mixed with 100 mL of methanol, ethanol, ethyl acetate, and distilled water separately and incubated with shaking for 48 h at room temperature (RT). After that they were filtered through Whatman No. 1 paper. Organic extracts were concentrated using a rotary evaporator at 40 °C, whereas aqueous extracts were freeze-dried.

Table 1. Propolis samples collected from Kazakhstan

Samples	Location
Pro1	Station 5: Zhanakorgan district
Pro2	Station 5: Kyzylorda district
Pro3	Station 5: Otrar district
Pro4	Station 5: Shieli district

2.2. DPPH Radical Scavenging Assay

Antioxidant activity was assessed using the DPPH assay as previously defined [13]. Sample solutions (40 μL) prepared in water were mixed with 160 μL of DPPH solution (0.4 mM) dissolved in ethanol. The reaction mixture was incubated in the dark at room temperature for 30 min. Water was used as the negative control, while BHA (Butylated hydroxyanisole) was

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used as the standard antioxidant. Absorbance was measured at 517 nm. The percentage inhibition was calculated from absorbance values, and IC_{50} values were calculated.

2.3. Total Phenolic Content Assay

TPC was determined using the Folin–Ciocalteu colorimetric assay with minor modifications to the method described by Clarke et al. [11]. Briefly, 20 µL of extract solution was mixed with 100 µL of 10% Folin–Ciocalteu reagent and incubated for 5 min. Subsequently, 80 µL of 7.5% sodium carbonate solution was added and the reaction mixture was incubated in the dark for 30 min at room temperature. Absorbance was measured at 765 nm using a BioTek Epoch microplate spectrophotometer. Gallic acid was used for preparation of the calibration curve and results were expressed as mg gallic acid equivalents (GAE)/g extract.

2.4. Total Flavonoid Content Assay

TFC was quantified using the aluminum chloride colorimetric method [12]. Extracts were reacted with 2% aluminum chloride solution and incubated at room temperature for 30 min. Absorbance was recorded at 415 nm, and TFC was expressed as mg quercetin equivalents (QE)/g extract using a quercetin calibration curve.

2.5. HUVEC Cell Culture and MTT Cytotoxicity Assay

HUVECs were cultured in DMEM containing 10% FBS and 1% penicillin–streptomycin at 37 °C and 5% CO_2, subcultured with Trypsin–EDTA, and seeded into 96-well plates. Cytotoxicity was assessed by the MTT assay. Propolis extracts prepared in 1% DMSO-containing culture medium were applied at 0.01–100 mg/mL for 24 h (n = 5). Cell viability was determined with the Vybrant MTT Cell Proliferation Assay Kit (Invitrogen) according to the manufacturer's instructions. Following 4 h incubation at 37 °C, absorbance was measured at 570 nm using an Epoch microplate reader (BioTek). Viability was expressed relative to untreated controls, and IC_{50} values were calculated using GraphPad Prism.

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2.6. Morphological Assessment of HUVEC Cells

HUVEC cells were treated with propolis extracts (10 mg/mL) for 24 or 48 h. Following treatment, cellular morphology was examined using an inverted microscope (Zeiss Axiovert A1, Carl Zeiss, Germany), and images were acquired with ZEN 3.1 (ZEN lite) imaging software (Carl Zeiss).

2.7. RNA Isolation

HUVEC cells were treated with propolis extract for 24 h prior to RNA isolation. Total RNA was extracted using Hydrozol reagent according to the manufacturer's protocol (A.B.T.™ RNA Purification Kit). Briefly, cell lysates were subjected to chloroform phase separation. The purified RNA pellets were dissolved in RNase-free water.

2.8. cDNA Synthesis

Complementary DNA (cDNA) was synthesized from total RNA using the GLPBIO SuperFast RT Master Mix for qPCR kit with gDNA Remover (Cat. No. GK10031) according to the manufacturer's instructions. Briefly, RNA (0.01–1 µg) was first treated with 2 µL of 5× gDNA Removal Mix in a final volume of 10 µL at 42 °C for 2 min. Subsequently, 4 µL of 5× SuperFast RT Mix and 6 µL of DEPC-treated ddH_2O were added to obtain a final reaction volume of 20 µL, followed by reverse transcription at 50 °C for 5 min.

2.9. Quantitative Real-Time PCR (qRT-PCR)

Gene expression was analyzed using synthesized cDNA and gene-specific primers targeting *GAPDH*, *TERF1*, *TERF2*, *TERT*, and *PINX1* (Table 2). *GAPDH* was used as the housekeeping gene. Each PCR reaction consisted of 2.5 µL BlasTaq™ 2× qPCR Master Mix, 0.5 µL each of forward and reverse primers (1 µM), 0.5 µL template cDNA (50 ng), and 1.5 µL nuclease-free water, giving a final volume of 5.5 µL. Amplification was carried out using an Applied Biosystems Real-Time PCR System with an initial enzyme activation at 95 °C for 3 min, followed by 40 cycles of denaturation at 95 °C for 15 s and annealing/extension at 60 °C for 1

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min. Relative fold changes were calculated from threshold cycle (Ct) values using the $2^{-\Delta\Delta Ct}$ method.

Table 2. Primers used for qRT-PCR analysis

Full gene name	Gene primers	Primer sequence (5′→3′)
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH-F	5'-GTCTCCTCTGACTTCAACAGCG-3'
	GAPDH-R	5'-ACCACCCTGTTGCTGTAGCCAA-3'
Telomeric Repeat Binding Factor 1	TERF 1-F	5'-CATGGAACCCAGCAACAAGACC-3'
	TERF 1-R	5'-CTGCTTTCAGTGGCTCTTCTGC-3'
Telomeric Repeat Binding Factor 2	TERF 2-F	5'-GTGGAAAAGCCACCCAGAGAAC-3'
	TERF 2-R	5'-TGCAAAGGCTGCCTCAGAATCC-3'
Telomerase Reverse Transcriptase	TERT-F	5'-GCCGATTGTGAACATGGACTACG-3'
	TERT-R	5'-GCTCGTAGTTGAGCACGCTGAA-3'
PIN2/TERF1-Interacting Telomerase Inhibitor 1	PINX 1-F	5'-TCCAGAGGAGAACGAAACCACG-3'
	PINX 1-R	5'-TACGTTCCACCTGCGTCTCAGA-3'

2.10. Statistical analyses

The statistics were performed using GraphPad Prism 10.0. Relative gene expression (ΔΔCt values) was analyzed by one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test (each treatment group vs. control). Data are presented as mean ± standard deviation (SD). Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Extraction Yield of Propolis

Extract yields were calculated as percentages (Table 3) and the yield values were between 6-12%. Total phenolic content was determined because phenolic compounds are closely associated with the biological activity of propolis extracts and may vary according to geographical and seasonal conditions.

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3.2. DPPH Radical Scavenging Activity

The antioxidant activity of propolis extracts is presented in Table 3. IC_{50} values ranged from 97.5 to 213.2 µg/mL, with lower values indicating stronger activity. Overall, the extracts showed moderate antioxidant activity compared with BHA.

3.3. Total Phenolic and Flavonoid Content

Total phenolic content ranged from 99.4 to 216.3 mg GAE/g extract, while total flavonoid content ranged from 6.9 to 13.6 mg QE/g extract (Table 3).

3.4. Cell Culture Findings

Propolis extracts were prepared at 100 mg/mL and diluted to 0.01–100 mg/mL. Based on MTT and microscopic findings, 10 mg/mL was selected for subsequent experiments. Cell morphology was evaluated after 24 and 48 h using a Zeiss Axio inverted microscope at 10× magnification (Figure 1). Propolis-treated HUVECs maintained normal morphology and proliferation without evident cellular stress. Given the IC_{50} values of 5.6–25.4 mg/mL, the extracts showed weak cytotoxicity, and no further anticancer analyses were performed. Experiments were conducted in five independent replicates (n = 5).

Table 3. Percentage yields, DPPH activity, TPC and TFC content of propolis extracts

Samples	Extracts	Yield %	DPPH• radical scavenging activity IC50 (µg/mL)	Total Phenolic Content (mg GAE/g extract ±SD)	Total Flavonoid Content (mg QE/g extract ±SD)	Cell Culture MTT results IC_{50} (mg/mL)
Pro1	Water	7%	195.3 ± 6.9	146.2 ± 5.6	7.9 ± 0.6	18.7
	Methanol	10%	148.79 ± 3.6	178.5 ± 8.6	9.9 ± 1.3	10.5
	Ethanol	11%	136.9 ± 4.1	173.4 ± 8.9	10.3 ± 1.6	7.5
	Ethyl acetate	8%	165.89 ± 6.3	161.7 ± 7.9	8.3 ± 1.0	19.5
Pro2	Water	6%	189.63 ± 6.9	153.1 ± 6.6	6.9 ± 0.5	20.2
	Methanol	9%	138.5 ± 6.9	179.8 ± 7.6	10.5 ± 0.9	10.1
	Ethanol	10%	135.9 ± 3.6	173.2 ± 7.3	11.9 ± 1.3	6.3
	Ethyl acetate	8%	154.6 ± 9.3	160.6 ± 6.9	8.3 ± 1.0	17.6
Pro3	Water	7%	169.5 ± 3.6	158.3 ± 6.9	7.1 ± 1.1	19.6
	Methanol	11%	141.3 ± 9.7	181.1 ± 7.6	12.4 ± 1.3	9.6
	Ethanol	11%	148.9 ± 6.3	196.7 ± 9.9	13.6 ± 1.1	5.6
	Ethyl acetate	9%	158.6 ± 9.3	161.7 ± 5.6	8.7 ± 1.0	16.3
Pro4	Water	7%	213.2 ± 6.9	99.4 ± 3.3	8.4 ± 0.9	25.4
	Methanol	10%	144.9 ± 6.3	126.7 ± 5.9	12.5 ± 0.8	9.1
	Ethanol	12%	97.5 ± 6.3	216.3 ± 8.6	12.3 ± 1.3	7.9
	Ethyl acetate	10%	118.6 ± 3.9	143.3 ± 5.6	7.7 ± 2.6	15.9
BHA: Reference substance for DPPH		-	10.1 ± 1.95	-	-	

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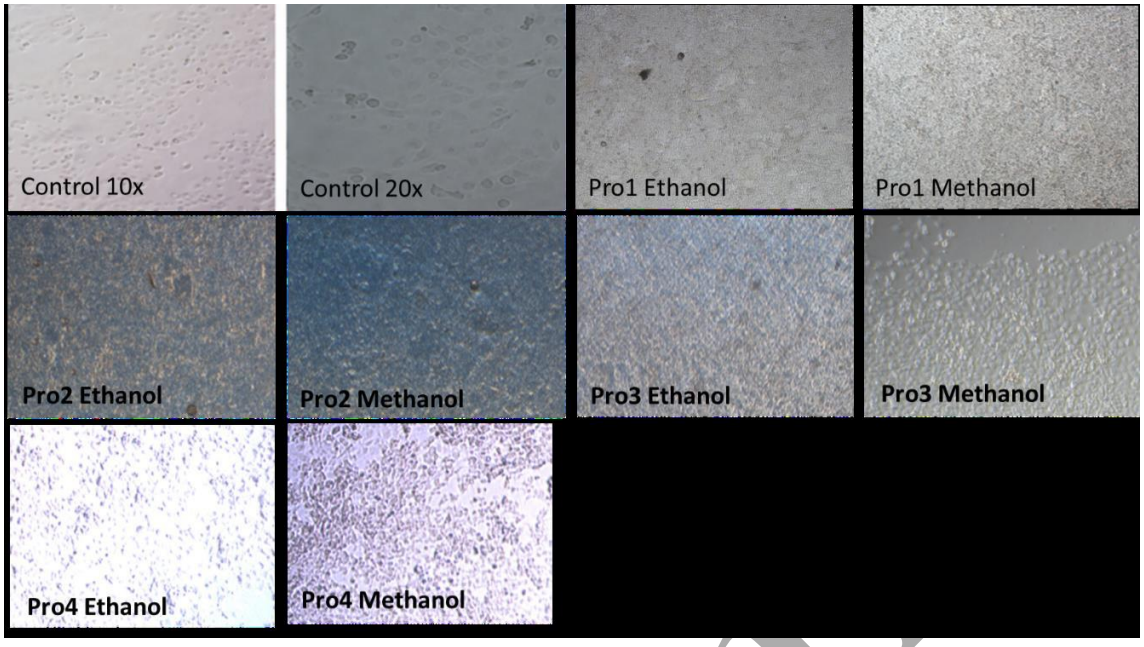


Figure 1. Representative microscopic images of control and propolis-treated HUVEC cells after 24 h of incubation. Images were obtained using a Zeiss Axio inverted microscope

3.5. Effects of Different Propolis Extracts on Telomere-Related Gene Expression in HUVEC Cells

Following cDNA synthesis, the relative expression of *TERT*, *TERF1*, *TERF2*, and *PINX1* was quantified by qRT-PCR using *GAPDH* as the reference gene and untreated cells as the calibrator. Propolis treatment altered telomere-related gene expression according to sample origin and extraction solvent (Table 4). *TERF1* showed the strongest response, particularly in methanolic extracts, with Pro3-Methanol producing the highest expression, followed by Pro2- and Pro4-Methanol. Methanolic extracts induced approximately 6–16-fold higher *TERF1* expression than the control (Table 4, Figure 2). *TERT* expression was highest in Pro3-Methanol and Pro4-Methanol, while *PINX1* showed moderate response and *TERF2* comparatively modest induction, with Pro3-Methanol producing the strongest responses.

Extraction solvent had a greater effect than geographical origin, with the general pattern Methanol> Ethanol> Ethyl acetate> Water, and Pro3 showed the strongest response. One-way

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ANOVA revealed significant differences for *TERF1* ($F = 8.404$, $p<0.0001$), *TERT* ($F = 5.670$, $p<0.0001$), and *TERF2* ($F = 4.363$, $p<0.001$), but not *PINX1* ($F = 1.214$, $p = 0.295$) (Table 5, Figure 2). Dunnett's test showed significantly higher TERT expression in several Pro3, Pro2, Pro1, and Pro4 groups ($p<0.05$), while *TERF1* upregulation was significant only for Pro2- and Pro3-Methanol extracts. No significant pairwise differences were detected for *TERF2* or *PINX1* after correction (Table 4, Figure 2).

Table 4. Relative expression of telomere-associated genes in HUVECs treated with propolis extracts for 24 h. Values are mean ± SD (n = 5), normalized to *GAPDH* and expressed relative to the untreated control (= 1.00). *p < 0.05, **p < 0.01, ***p < 0.001 versus control (one-way ANOVA with Dunnett's test).

Sample	Water	Ethanol	Methanol	Ethyl acetate
***TERT* gene expression values (Dunnett's test vs. control)**				
Propolis 1	1.48 ± 0.22	1.62 ± 0.19**	2.61 ± 0.28	1.86 ± 0.20
Propolis 2	1.34 ± 0.17**	2.08 ± 0.23	2.29 ± 0.21	1.77 ± 0.18**
Propolis 3	1.92 ± 0.21***	2.47 ± 0.25***	3.21 ± 0.34***	2.53 ± 0.29
Propolis 4	1.86 ± 0.18	2.35 ± 0.24**	2.89 ± 0.31	2.41 ± 0.25
***TERF2* gene expression values (Dunnett's test vs. control)**				
Propolis 1	1.34 ± 0.18	2.24 ± 0.24	2.91 ± 0.31***	1.82 ± 0.19
Propolis 2	1.05 ± 0.12	2.03 ± 0.22	1.94 ± 0.21	1.37 ± 0.15
Propolis 3	1.61 ± 0.18	2.27 ± 0.23	3.08 ± 0.33***	2.06 ± 0.22
Propolis 4	0.82 ± 0.10	1.02 ± 0.13	1.26 ± 0.16	1.11 ± 0.12
***PINX1* gene expression values (Dunnett's test vs. control)**				
Propolis 1	0.81 ± 0.09	1.34 ± 0.16	1.62 ± 0.19	1.42 ± 0.17
Propolis 2	0.94 ± 0.11	1.51 ± 0.18	1.46 ± 0.17	1.13 ± 0.15
Propolis 3	1.68 ± 0.19	3.76 ± 0.35***	4.62 ± 0.41***	2.51 ± 0.24
Propolis 4	1.72 ± 0.20	3.04 ± 0.29***	4.08 ± 0.37***	2.44 ± 0.23
***TERF1* gene expression values (Dunnett's test vs. control)**				
Propolis 1	2.41 ± 0.26	4.55 ± 0.41***	6.91 ± 0.54***	2.78 ± 0.30
Propolis 2	4.23 ± 0.39***	9.63 ± 0.81***	13.81 ± 1.05***	5.12 ± 0.48***
Propolis 3	3.76 ± 0.33***	11.26 ± 0.95***	15.83 ± 1.18***	6.08 ± 0.52***
Propolis 4	3.64 ± 0.30***	7.11 ± 0.62***	12.35 ± 0.94***	5.41 ± 0.46***

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Table 5. One-way ANOVA results of genes

Gene	F value	p value	Significance
TERT	5.670	0.000002	Significant
TERF1	8.404	1.01×10^{-8}	Significant
TERF2	4.363	0.000043	Significant
PINX1	1.214	0.295	Not Significant

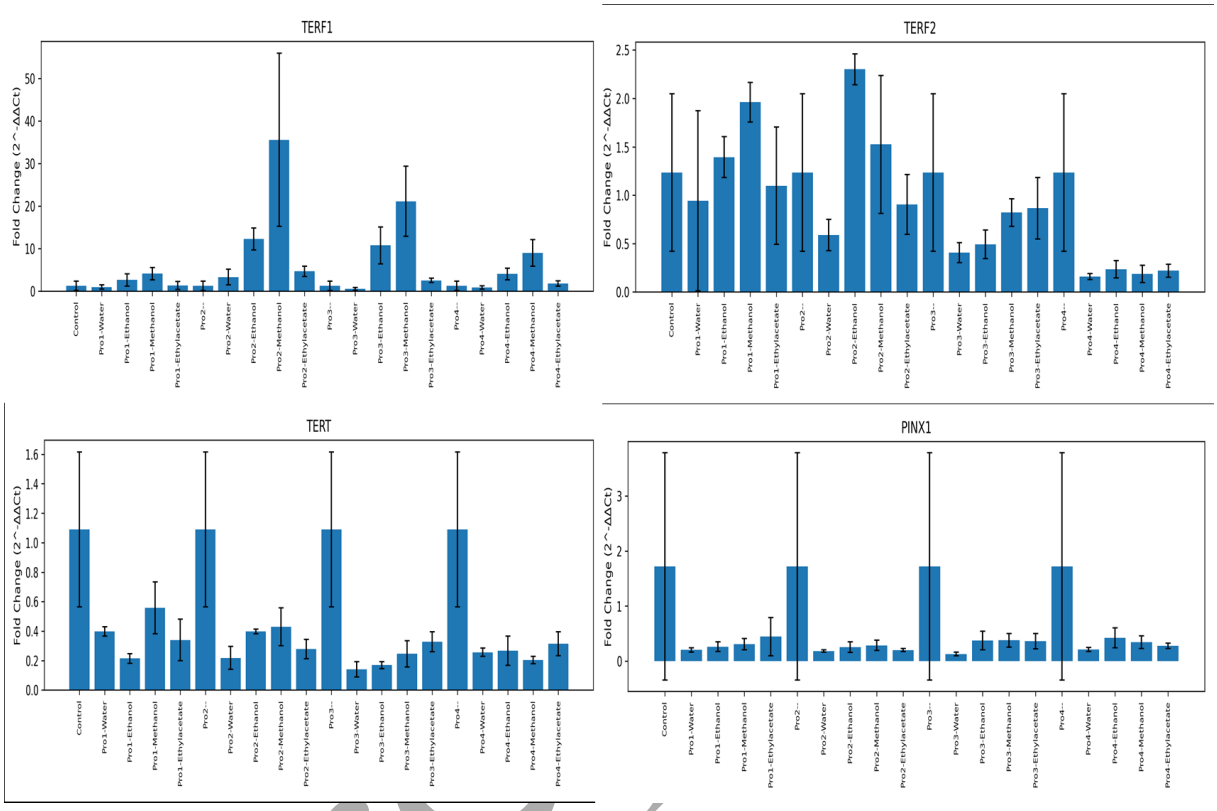


Figure 2. Relative expression levels of telomere-associated genes (*TERT*, *TERF1*, *TERF2* and *PINX1*) in HUVEC cells following 24-hour treatment with four propolis samples extracted using different solvents

4. Discussion

The present data suggest that extraction solvent markedly changed the biological activity of extracts. Methanolic and ethanolic extracts generally showed higher phenolic and flavonoid contents and stronger antioxidant activity than aqueous extracts, whereas methanolic extracts induced the strongest changes in the expression of telomere-associated genes in HUVECs. Methanol and ethanol efficiently recover compounds such as chrysin, galangin, CAPE, and quercetin derivatives, while water preferentially extracts highly polar constituents. The

association between total phenolic content and DPPH activity further supports the contribution of phenolics to propolis antioxidant capacity.

Oxidative stress promotes telomere shortening and endothelial senescence, as guanine-rich telomeric DNA is particularly susceptible to oxidative damage. Thus, the higher antioxidant activity of methanolic and ethanolic extracts may partly explain their stronger effects on telomere-related genes. By reducing oxidative damage, propolis antioxidants may help preserve telomere integrity and regulate telomere-maintenance pathways.

TERF1 showed the most pronounced response, with methanolic extracts producing the highest induction across propolis origins. Pro2- and Pro3-Methanol increased *TERF1* expression by approximately 14–16-fold relative to the control, and Dunnett's test confirmed significant upregulation in these groups. As a core shelterin component, *TERF1* binds double-stranded telomeric DNA and contributes to telomere length homeostasis and chromosome-end protection. Although excessive *TERF1* can restrict telomerase accessibility, moderate induction may support telomere stabilization under oxidative stress.

The strong *TERF1* response to methanolic extracts may reflect their greater recovery of flavonoids and phenolic acids, including CAPE, chrysin, galangin, pinocembrin, quercetin, and caffeic acid derivatives. These compounds may provide a potential mechanistic link between antioxidant activity and *TERF1* regulation.

Although Pro3 generally showed the strongest responses, samples followed a similar activity pattern (Methanol> Ethanol>Ethyl acetate >Water), indicating that extraction efficiency was a major determinant of biological activity.

TERT expression was also strongly affected by propolis treatment, with methanolic extracts consistently producing the highest levels. Pro3-Methanol showed the strongest increase (3.21 ± 0.34-fold), followed by Pro4-Methanol (2.89 ± 0.31-fold), whereas water extracts showed weaker and ethanol and ethyl acetate intermediate responses. Significant differences among

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groups and higher expression in several Pro3-treated groups were confirmed statistically. Telomerase contributes to chromosome stability by compensating for progressive telomere shortening [3,4]. Although its expression is limited in most differentiated cells, endothelial cells retain measurable activity that contributes to vascular homeostasis. Oxidative stress may suppress *TERT* transcription, whereas antioxidant phytochemicals may restore telomerase-related signaling through Nrf2, PI3K/Akt, and SIRT1. Thus, increased *TERT* expression may reflect improved redox balance rather than uncontrolled proliferation.

The association between antioxidant activity and *TERT* expression further supports this interpretation, as the methanolic and ethanolic extracts showed the lowest DPPH IC_{50} values and highest phenolic contents. *TERF2* showed a more moderate but generally consistent increase, particularly with Pro1- and Pro3-Methanol. Although the overall group effect was significant, Dunnett's analysis detected no significant pairwise differences. As a key shelterin component, *TERF2* stabilizes telomeric structures and prevents inappropriate DNA damage responses; its modest modulation may therefore reflect preservation of telomere homeostasis rather than excessive alteration of shelterin function.

In contrast, *PINX1* showed a moderate response, and overall differences among groups were not statistically significant. *PINX1* is an endogenous regulator of telomerase through its interaction with *TERT*, but it also contributes to chromosome stability and telomere architecture. Therefore, the simultaneous increase in *TERT* and *PINX1* observed in some methanol-treated groups may represent a compensatory mechanism that permits telomerase activation while preventing excessive telomere elongation. Such coordinated regulation may reflect maintenance of telomere homeostasis rather than contradictory biological effects.

Overall, extraction solvent exerted a greater influence on telomere-related gene expression than geographical origin, with the consistent hierarchy of Methanol>Ethanol>Ethyl acetate >Water. Nevertheless, considerable variation among Pro1–Pro4 was observed, which is consistent with

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the known influence of botanical origin, geography, climate, and local vegetation on propolis composition [14–16]. Pro3 generally produced the strongest responses, particularly for *TERF1* and *TERT*. Its activity may be related to a richer profile of flavonoids and phenolic compounds capable of modulating PI3K/Akt, MAPK, Nrf2, FOXO, and NF-κB pathways, which interact with telomerase regulation and cellular senescence [17].

The coordinated changes in *TERF1*, *TERT*, *TERF2*, and *PINX1* suggest that propolis may not act on a single telomere-associated target but modulates multiple components involved in telomere protection and endothelial homeostasis. Alterations in shelterin proteins may accompany telomeric remodeling under oxidative stress, as the shelterin complex functions as an integrated regulatory network [18]. The simultaneous induction of *TERT* and *PINX1* may further reflect negative-feedback regulation following telomerase activation, supporting a balanced rather than excessive telomere response. In this case, the present data indicate that propolis may act as a cellular modulator, fine-tuning telomere-associated pathways according to its phytochemical composition.

Several limitations should be acknowledged. First, only mRNA expression was measured; protein abundance and enzymatic activity were not assessed. Second, telomerase activity was not directly determined using the TRAP assay; therefore, increased *TERT* expression cannot be considered definitive evidence of increased telomerase activity. Third, telomere length was not measured by qPCR or terminal restriction fragment analysis. Accordingly, the functional consequences of the observed transcriptional changes remain to be experimentally validated.

5. Conclusion

Finally, propolis extracts may affect the expression of key telomere-associated genes in HUVEC cells, with methanolic extracts generally producing the strongest responses. The findings further indicate that extraction solvent had a greater influence on telomere-related gene expression than geographical origin, highlighting the importance of extraction strategy in

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determining the biological activity of propolis. Future studies should integrate transcriptomic, proteomic, and functional approaches, including TRAP assays, shelterin protein analyses, ROS measurements, and telomere-length determination, to clarify the mechanisms underlying propolis-mediated telomere regulation. LC-MS/MS or UHPLC-QTOF-MS profiling could further link specific bioactive compounds to telomere-related responses. Overall, these findings identify solvent-specific propolis extracts as potential modulators of telomere-associated gene expression, although further mechanistic and functional validation is required before therapeutic or preventive applications can be considered.

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Authors' Contribution

Conceptualization: ZS, SDD. Data curation: ZS, SDD, SK, YB. Formal analysis: ZS, SK, AO. Methodology: ZS, SDD, SK, AO. Software: SK, YB, TD. Visualization: SDD, SK, TD. Investigation: ZS, SDD, SK. Writing – original draft: SDD, SK. Writing – review and editing: ZS, SDD, SK.

Ethics

Ethics committee approval is not required for this study.

Conflict of Interest

No potential conflicts of interest relevant to this article were reported.

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Data Availability

The data that support the findings of this study are available on request from the corresponding author.

Declaration on the Use of AI

During the preparation and revision of this article, the authors used Claude (Anthropic) to improve the clarity, grammar, and readability of the English text, as well as to enhance the visual presentation of the figures. In the revision phase, Claude was also used to check the text for grammatical accuracy, formatting, and terminology. All scientific content, data analysis, interpretations, and conclusions were generated, verified, and approved by the authors. The authors bear full responsibility for the content of the publication.

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