# Identification of Telomerase-activating Blends From Naturally Occurring Compounds

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## ABSTRACT

**Context** • Telomeres are repeated deoxyribonucleic acid (DNA) sequences (TTAGGG) that are located on the 5' ends of chromosomes, and they control the life span of eukaryotic cells. Compelling evidence has shown that the length of a person's life is dictated by the limited number of times that a human cell can divide. The enzyme telomerase has been shown to bind to and extend the length of telomeres. Thus, strategies for activating telomerase may help maintain telomere length and, thus, may lead to improved health during aging.

**Objective** • The current study intended to investigate the effects of several natural compounds on telomerase activity in an established cell model of telomere shortening (ie, IMR90 cells).

**Design** • The research team designed an in vitro study.

**Setting** • The study was conducted at Roskamp Institute in Sarasota, FL, USA.

**Intervention** • The tested single compounds were (1) α-lipoic acid, (1) green tea extract, (2) dimethylaminoethanol L-bitartrate (DMAE L-bitartrate), (3) N-acetyl-L-cysteine hydrochloride (HCL), (4) chlorella powder, (5) L-carnosine, (6) vitamin $D_3$, (7) rhodiola PE 3%/1%, (8) glycine, (9) French red wine extract, (10) chia seed extract, (11) broccoli seed extract, and (12) *Astragalus* (TA-65). The compounds were tested singly and as blends.

**Outcome Measures** • Telomerase activity for single compounds and blends of compounds was measured by the TeloTAGGG telomerase polymerase chain reaction (PCR) enzyme-linked immunosorbent assay (ELISA). The 4 most potent blends were investigated for their effects on cancer-cell proliferation and for their potential effects on the cytotoxicity and antiproliferative activity of a chemotherapeutic agent, the topoisomerase I inhibitor topotecan. The benefits of 6 population doublings (PDs) were measured for the single compounds, and the 4 blends were compared to 3 concentrations of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

**Results** • Certain of the compounds increased telomerase activity, and combinations of the top-ranking compounds were able to increase telomerase activity significantly, from 51% to 290%, relative to controls.

**Conclusions** • The results have confirmed that many naturally occurring compounds hold the potential to activate telomerase and that certain of those compounds have demonstrated synergistic effects to produce more potent blends. Given the relationship between telomere shortening, aging, and the decline of tissue function, it is reasonable to hypothesize that such telomerase-activating blends may have health-promoting benefits, particularly in relation to aging-associated conditions. Further investigation of such blends in human studies that are designed to evaluate safety and the effects on telomere length are thus warranted. (*Altern Ther Health Med.* 2016;22(S2):6-14.)

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Telomeres are repeated deoxyribonucleic acid (DNA) sequences (TTAGGG) located on the 5' ends of human chromosomes. The length of the telomere in human somatic cells is believed to be heterogeneous, ranging from 5 to 20 kb and is determined by a person's age, the organ examined, and the number of divisions that each of its cells has endured.[1]

A telomere can lose up to 200 bases with each DNA replication cycle. The progressive shortening of telomeres that follows is one of the molecular mechanisms associated with aging, because critically short telomeres trigger chromosome senescence and loss of cell viability.[1-3] Telomere shortening occurs as a result of the progressive loss of these repeated nucleotide sequences that takes place over many replication cycles. That loss can be stopped and even reversed by the enzyme telomerase.[4,5]

Telomerase is a ribonucleoprotein enzyme that lengthens the telomere by extending the DNA termini. Telomere length has also been found to decrease with age in humans, suggesting a role between telomere length and the fate of cells and the decline of tissue function that eventually affects mortality.[6]

It has been suggested that shelterin, a protein complex with DNA remodeling activity, acts together with several associated DNA-repair factors to fold the telomere's end into a properly capped structure, thereby protecting chromosomes' ends.[7] It is also known that cellular senescence can be triggered by the accumulation of too many uncapped telomeres and that the repair of critically short telomeres by telomerase or recombination is limited in most somatic cells. Therefore, the length of the telomere repeats can regulate the proper capping of the ends of the chromosomes and protect them.

Telomerase affects longevity by preventing premature telomere erosion, with that loss exemplified by human diseases with mutations in telomerase components. Individuals with such mutations often develop premature dysfunction of adult stem cells and show signs of decreased longevity due to accelerated rates of telomere shortening.[2,8-12]

Although no evidence is available that telomerase drives the oncogenic process, it is permissive and required for the sustained growth of cancers. Therefore, almost all human cancers show telomerase activation as a hallmark process, and it is the likely mechanism that allows unlimited cell proliferation of tumor cells.[13] However, telomere erosion has also been documented in hyperproliferative disease states, as a condition strongly associated with increased cancer risk. Short telomeres have a higher predisposition to induce chromosome rearrangements that can lead to cancer, and it has been suggested that short telomeres may actually be a cause of cancer[14,15] and may precede reactivation of telomerase.[5,16,17]

Two studies have suggested that the activation of telomerase is sufficient to delay aging and increase the lifespan in normal aging and accelerated aging mice, without any increase in cancer incidence.[18-20] Many more studies have suggested that the reactivation of telomerase in adult or aged organisms has a similar positive effect on the delay of aging and that the mechanism may be independent of its role in cancer proliferation where it is aberrantly expressed.

Given the projected increase in life span of the human population, healthy aging might be facilitated by approaches that maintain telomere length. A few compounds are already commercially available, in the form of dietary supplements, that claim to maintain or repair telomere length and that are associated with increased longevity through telomerase activation.[18]

The present study was designed to investigate the effects of a variety of natural compounds on telomerase activity, followed by an evaluation of whether combinations of the most potent telomerase modulators might enhance the performance of any single modulator. Also, given the tight link between telomerase activity, cellular senescence, and cancer, the effects of the 4 most effective blends for the increase of telomerase activity were investigated regarding their effects on tumor cell proliferation and survival in response to a chemotherapeutic agent, to assess any potential cancer risk associated with their promotion of telomerase activity.

## METHODS

The effects of the current study's compounds and their combinations on telomerase activity were tested in vitro using primary human IMR90 cells. The cells were treated with each compound for 15 population doublings (PDs) and compared to the commercially available telomerase activator, *Astragalus* (TA-65), which has been shown to have beneficial effects by increasing telomerase activity.[18,21-23] The 4 blends that showed the highest performance were also compared in the same assay to the effects of omega-3 fatty acids (FAs), which have been shown to increase telomere length in a human study.[24]

### Cells

The telomeres of IMR90 cells shorten by an average of 70 base pairs per division.[25] Those cells were chosen for the current study. IMR90 cells were purchased from ATCC (Rockville, MD, USA) and the cells were grown in Eagle's minimum essential medium (EMEM) from the American Type Culture Collection (ATCC) (Manassas, VA, USA), supplemented with 10% fetal bovine serum in a 5% $CO_2$, humidified environment at 37°C.

### Procedures

**Single Compounds.** The tested compounds were (1) α-lipoic acid, (1) green tea extract, (2) dimethylaminoethanol L-bitartrate (DMAE L-bitartrate), (3) *N*-acetyl-L-cysteine hydrochloride (HCL), (4) *Chlorella* powder, (5) L-carnosine, (6) vitamin D₃, (7) rhodiola PE 3%/1%, (8) glycine, (9) French red wine extract, (10) chia seed extract, (11) broccoli seed extract, (12) *Astragalus* extract, and (13) TA-65, which acted as the positive control. TA-65 has been previously described as a compound capable of increasing average telomere length by turning on the *hTERT* gene, which activates telomerase.

All of the compounds tested were provided by Enzymedica (Venice, FL, USA). Eicosapentaenoic acid (EPA)—20:5, *n*-3; docosahexaenoic acid (DHA)—22:6, *n*-3;

and delipidated and charcoal-treated fetal bovine serum were all purchased from Sigma-Aldrich (St Louis, MO, USA).

**Blends of Compounds.** Eight compounds were tested: (1) BL-1: rhodiola PE 3%/1%, sulforaphane glucosinolate (SGS) broccoli seed extract, *Astragalus* extract, L-carnosine, *N*-acetyl-L-cysteine HCL, and vitamin $D_3$; (2) BL-2: rhodiola PE 3%/1%, SGS broccoli seed extract, *Astragalus* extract, L-carnosine, *N*-acetyl-L-cysteine HCL, vitamin $D_3$, and an enzyme blend containing xylanase, pectinase, hemicellulase, cellulase TB, protease, and catalase; (3) BL-3: *Astragalus* extract, SGS broccoli seed extract, rhodiola PE 3%/1%, and vitamin $D_3$; (4) BL-4: *Astragalus* extract, SGS broccoli seed extract, and rhodiola PE 3%/1%; (5) BL-5: *Chlorella* powder, *Astragalus* extract , rhodiola PE 3%/1%, *N*-acetyl-L-cysteine HCL, SGS broccoli seed extract, L-carnosine, and vitamin $D_3$; (6) BL-6: rhodiola PE 3%/1%, *N*-acetyl-L-cysteine HCL, L-carnosine, SGS broccoli seed extract, and vitamin $D_3$; (7) BL-7: *Chlorella* powder, glycine, and vitamin $D_3$; and (8) BL-8: rhodiola PE 3%/1%, L-carnosine, SGS broccoli seed extract, L-carnosine, *Chlorella* powder, and TA-65. Enzymedica currently markets BL-3 as Telomere Plus.

**Lactate Dehydrogenase Assay.** Regarding the cell treatments and sample collection, all compounds were first tested for their toxicity by measuring the release of lactate dehydrogenase (LDH) from Roche (Indianapolis, IN, USA) at a dose range of 50 μg/mL to 1000 μg/mL. The cells were then treated with each compound for 15 PDs, unless stated otherwise. Overall, the cells were passaged into several flasks and allowed to attach for a few hours, before subsequently being treated for 72 hours with the test compound. After the 72 hours, the cells were detached with TrypLE (Invitrogen, Grand Island, NY, USA). Half of the cells were washed and flash frozen in a -80°C freezer for analyses, and the other half were seeded in a fresh flask and treated with the same compound for 72 hours. The same paradigm was repeated for up to 15 PDs.

**Sample Preparation.** After the last PD treatment and sample collection (PD-15), cells from all passages, including previously frozen passages, were resuspended in a 150-L lysis reagent provided in the telomeric repeat amplification protocol (TRAP) assay, precooled on ice, and incubated for 30 minutes on ice. The samples were prepared as per the manufacturer's recommendation; briefly, cell lysates were centrifuged at 16 000 × G for 20 minutes at 2°C to 8°C. The supernatant was carefully collected, and the protein content was subsequently quantified using a bicinchoninic acid (BCA) assay.

### Outcome Measures

**TRAP Assay.** The telomerase activity was measured by a TeloTAGGG telomerase polymerase chain reaction (PCR) enzyme-linked immunosorbent assay (ELISA) assay from Roche. It is a photometric enzyme immunoassay that is used for the detection of telomerase activity using TRAP. The procedure was performed in accordance with the manufacturer's instructions. Briefly, a first-step PCR elongation and amplification was followed by an ELISA detection step. For each reaction, the negative control was a heat-treated sample, and the positive control was a human embryonic kidney (HEK) cell extract that was provided in the kit.

**Cytotoxicity and Cell Proliferation Screening.** The 4 blends that were found to be most potent for telomerase activity—BL-1, BL-2, BL-3, and BL-4—were also investigated for their effects on cancer cell proliferation. Adenocarcinomic, human, alveolar basal epithelial cells (A549 cells) were grown in Dulbecco's modified Eagle medium (DMEM) from ThermoFisher Scientific (Grand Island, NY, USA), which was supplemented with 10% fetal bovine serum and 1× penicillin/streptomycin. In addition, the potential impacts of BL-1, BL-2, BL-3, and BL-4 on the cytotoxicity and the antiproliferative activity of a chemotherapeutic agent, the topoisomerase I inhibitor topotecan, was investigated at a dose range of 0.1 μM to 10 μM of topotecan.

Cytotoxicity was monitored by measuring the release of LDH in the culture media, following 48 hours of treatment with a dose range of topotecan. In addition, the number of live cells adherent to the cell culture wells was quantified by measuring the cleavage of the tetrazolium salt WST-1 to formazan by cellular mitochondrial dehydrogenases (Biovision, Milpitas, CA, USA), to determine the effect of the different treatments on cellular proliferation.

**Preparation of Albumin-bound FAs.** Cultured IMR90 cells were incubated with albumin-bound EPA—20:5, *n*-3; sigma or DHA—22:6, *n*-3; sigma, for 72 hours in EMEM containing 10% delipidated and charcoal-treated, fetal bovine serum to minimize interference from serum FAs and to obtain a better-defined system.

Briefly, the EPA and DHA were resuspended in ethanol and stored at -20°C under nitrogen. The concentrations of DHA and EPA to be tested were evaporated to dryness under reduced pressure and the FAs were complexed with the depleted bovine serum albumin in a 1:1 molar ratio according to the method described by Mahoney.[26] The final concentrations were EPA 25 μM/DHA 2.5 μM; EPA 2.5 μM/DHA 0.25 μM; and EPA 0.25 μM/DHA 0.025 μM. The treatment was carried out for 6 PDs and compared with the 4 most potent blends, BL-1, BL-2, BL-3, and BL-4.

### Statistical Analysis

A matched-pairs *t* test was used to assess significant changes between 2 time points. Statistical significance was set at α < .05 for all statistical analyses. Statistical significance is indicated with a notation in graphs.

## RESULTS
### LDH Assay

The results revealed that 24 hours of treatment with 200 μg/mL of DMAE L-bitartrate, *N*-acetyl-L-cysteine HCL, *Chlorella* powder, or L-carnosine did not show any toxicity; thus, that dose was selected for those 4 compounds.

The TA-65, vitamin $D_3$, α-lipoic acid, green tea extract, and rhodiola PE 3%/1% were toxic at doses higher than 50 μg/mL; therefore, that concentration was used for those compounds.

In addition, the French wine extract showed some toxicity at a dose of 500 μg/mL, and *Astragalus* extract

**Figure 1.** Mean Value of Telomerase Activity With Time at PD-8 and PD-15 Compared With PD-2, for α-Lipoic Acid, Green Tea Extract, DMAE L-bitartrate, *N*-acetyl-L-cysteine HCL, *Chlorella* Powder, TA-65, and L-Carnosine
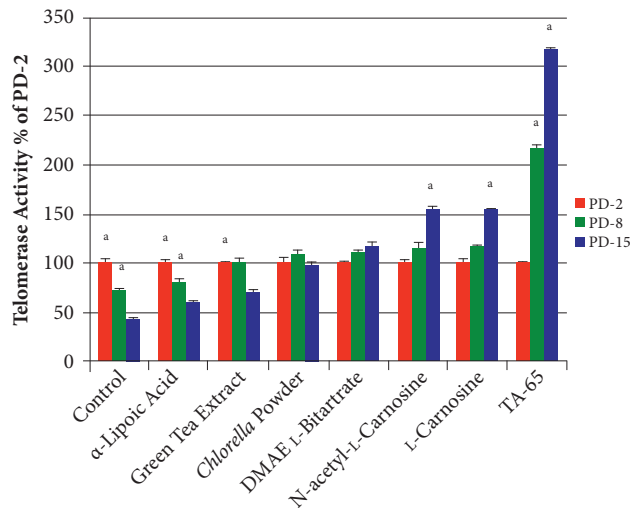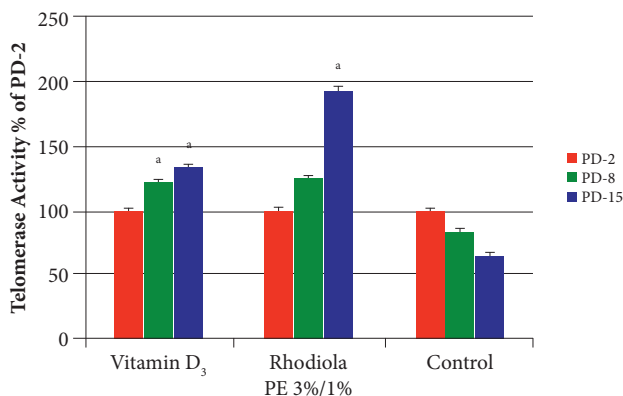


Note: Each error bar was constructed using 1 standard error from the mean. Statistical significance was set at α = .05 for all statistical analyses.

[a]For L-carnosine, *P* = .005 and for *N*-acetyl-L-cysteine HCL, *P* = .007 for PD-2 to PD-15, both significant increases. For TA-65, *P* = .0023 and *P* = .007, for PD-2 to PD-8 and for PD-2 to PD-15, respectively, both significant increases. For α-lipoic acid, *P* = .042 for PD-2 to PD-8 and *P* = .021 for PD-8 to PD-15, both significant decreases. For green tea extract, *P* = .023 for PD-2 to PD-15, a significant decrease.

Abbreviations: DMAE, dimethylaminoethanol; HCL, hydrochloride.

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**Figure 2.** Mean Value of Telomerase Activity With Time at PD-8 and PD-15 Compared With PD-2 for Vitamin D$_3$ and Rhodiola PE 3%/1%



Note: Each error bar was constructed using 1 standard error from the mean. Statistical significance was set at α = .05 for all statistical analyses.

[a]For vitamin D$_3$, *P* = .036 for PD-2 to PD-8 and *P* = .047 for PD-2 to PD-15, both significant increases. For rhodiola PE 3%/1%, *P* = .066 for PD-2 to PD-15, an increase indicating a trend toward significance.

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showed toxicity at a dose of 1000 μg/mL; therefore, those 2 compounds were tested at 250 μg/mL and 750 μg/mL, respectively. Glycine, chia seed extract, and broccoli seed extract did not show any toxicity at the highest dose tested, 1000 μg/mL, after 24 hours of treatment; therefore, those compounds were subsequently tested at 1000 μg/mL.

The chia seed and *Astragalus* extract were poorly soluble and, therefore, the powder was crushed using a pestle and mortar, and subsequently resuspended in ethanol and dimethyl sulfoxide (DMSO), respectively, before finally being sonicated for 1 minute. The soluble fraction was collected and then filtered via a 0.22 μm filter before use. Therefore, although the starting dose of the powder was 50 μg/mL, some of the powder remained undissolved; thus, the final dose was an estimate (data not shown).

**Single Compounds**

To investigate the telomerase activity in the single compounds, the research team selected passages 2, 8 or 10, and 15 to compare the telomerase activity profile. The data showed that telomerase activity was reduced with time in the control cells that were not treated, with *P* = .045 when comparing PD-2 to PD-8 and *P* = .012 when comparing PD-2 to PD-15 (Figure 1). That finding was as the research team expected, reflecting a shortening of the telomeres of the primary IMR90 cells because those cells have a finite life span.

The telomerase assay detected a significant increase in the telomerase activity for the positive control, TA-65, with *P* = .0023 and *P* = .007 when comparing PD-2 to PD-8 and PD-2 to PD-15, respectively (Figure 1).

**Compounds Showing Evidence for Increasing Telomerase Activity.** When comparing PD-2 to PD-8 for L-carnosine, *N*-acetyl-L-cysteine, and DMAE L-bitartrate, no significant differences were detected. However, when comparing PD-2 to PD-15, those 2 compounds, a significant increase in telomerase activity was observed, with *P* = .005 and *P* = .007, respectively (Figure 1). No trend existed for DMAE L-bitartrate to increase telomerase activity when comparing PD-2 to PD-15, and that measure did not reach statistical significance, with *P* = .089 (Figure 1).

Treatment with vitamin D$_3$ showed significantly increased telomerase activity when comparing PD-2 to PD-8, with *P* = .036, and a marginally significant increase when comparing PD-2 to PD-15, with *P* = .047 (Figure 2).

**Figure 3.** Mean Value of Telomerase Activity With Time at PD-10 and PD-15 Compared With PD-2 for Glycine, French Wine Extract, Chia Seed Extract, *Astragalus* Extract, and Broccoli Seed Extract
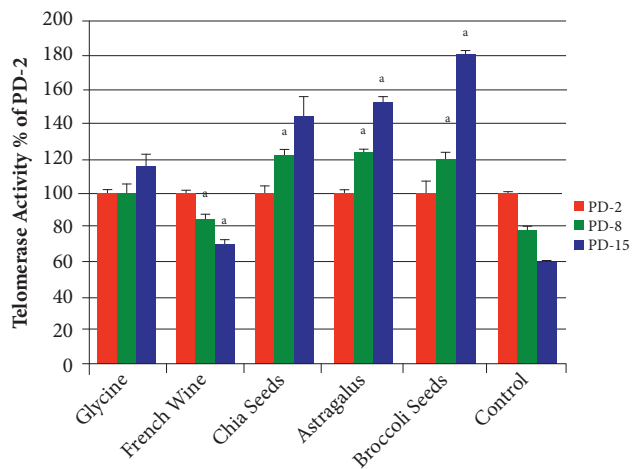


Note: Each error bar was constructed using 1 standard error from the mean. Statistical significance was set at α = .05 for all statistical analyses.

ᵃFor *Astragalus*, P = .0068 for PD-2 to PD-10 and P = .0014 for PD-2 to PD-15, both significant increases. For broccoli seed extract, P = .018 for PD-2 to PD-15, a significant increase. For chia seed extract, P = .048 for PD-2 to PD-10, a marginally significant increase, but the effect was lost when comparing PD-2 with PD-15, with P = .07. For French wine extract, P = .021 for PD-2 to PD-10 and P = .003 for PD-2 to PD-15, both significant decreases.

**Figure 4.** Effect of Blends BL-1 to BL-8 on Telomerase Activity: Mean Value With Time at PD-8 and PD-15 Compared With PD-2



Note: Each error bar was constructed using 1 standard deviation from the mean. Statistical significance was set at α = .05 for all statistical analyses.

ᵃFor BL-1, P = .022 for PD-2 to PD-8 and P = .044 for PD-2 to PD-15, both significant increases. For BL-2, P = .019 for PD-2 to PD-8 and P = .002 for PD-2 to PD-15, both significant increases. For BL-3, P = .041 for PD-2 to PD-8 and P = .011 for PD-2 to PD-15, both significant increases. For BL-4, P = .034 for PD-2 to PD-8 and P = .011 for PD-2 to PD-15, both significant increases. For BL-8, P = .028, a significant increase for PD-2 to PD-8 only. For BL-5, P = .016 for PD-2 to PD-15, a significant increase. For BL-6, P = .016 for PD-2 to PD-15, a significant increase. For BL-7, P = .013 for PD-2 to PD-15, a significant increase.
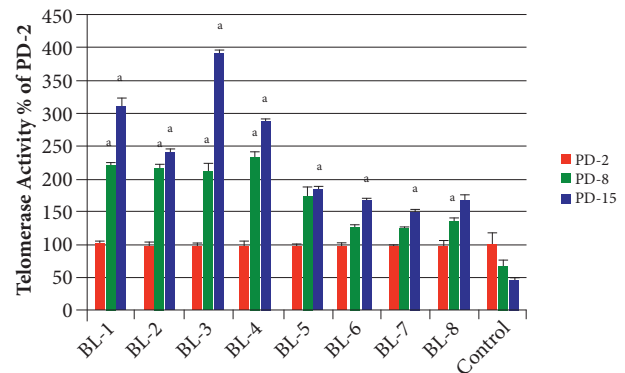
The rhodiola PE 3%/1% treatment showed a trend toward an increase in telomerase activity but only for the comparison of PD-2 to PD-15, with P = .066. No significant change was detected when comparing PD-2 to PD-8 (Figure 2).

In addition, the results from the telomerase assay detected a significant increase in telomerase activity with the *Astragalus* extract treatment, with P = .0068 when comparing PD-2 to PD-10 and P = .0014 when comparing PD-2 to PD-15 (Figure 3). When comparing PD-2 to PD-10 for the broccoli seed extract, no significant differences were detected; however, when comparing P-2 to P-15, a significant increase in telomerase activity was observed, with P = .018 (Figure 3).

The chia seed extract showed a marginally significant increase in telomerase activity, with P = .048 when comparing PD-2 to PD-10, but that effect was lost when comparing PD-2 to PD-15, with P = .07 (Figure 3).

**Compounds Showing Evidence for Stabilizing Telomerase Activity.** *Chlorella* powder did not have any effect on telomerase activity but also did not show any evidence of telomere shortening, suggesting that the *Chlorella* powder maintained the same level of telomerase activity with time (Figure 1). A similar effect was seen with the glycine treatment, suggesting a stabilization of telomerase activity with time (Figure 3).

**Compounds Showing Evidence for Decreasing Telomerase Activity.** Cells treated with α-lipoic acid showed a similar profile to the control sample, denoting reduced telomerase activity and, therefore, shorter telomeres. A significant decrease in telomerase activity appeared for that compound when comparing PD-2 to PD-8 and PD-2 to PD-15, with P = .042 and P = .021, respectively (Figure 1).

Green tea treatment did not show any effect on telomerase activity from PD-2 to PD-8, but from PD-2 and PD-15, a clear reduction in telomerase activity occurred, with P = .023 (Figure 1). Similarly, treatment with French wine extract revealed a clear decrease in telomerase activity when comparing PD-2 to PD-10 and PD-2 to PD-15, with P = .021 and P = .003, respectively (Figure 3).

**Blends of Compounds**

After the initial screening with the individual compounds, the research team tested 8 proprietary blends (BL-1 through BL-8) from the best performing compounds. The results from the telomerase assay detected a significant increase in telomerase activity with the BL-1 blend when comparing PD-2

**Figure 5.** Mean Value of Telomerase Activity With Time at PD-2, PD-8, and PD-15 Compared With PD- 1, for the Best 4 Blends Compared With EPA/DHA
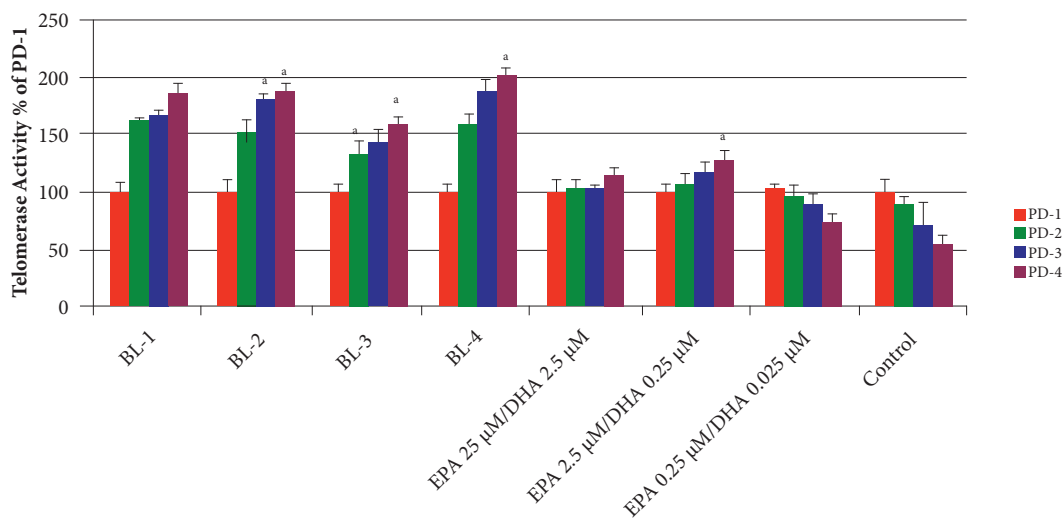


Note: Each error bar was constructed using 1 standard deviation from the mean. Statistical significance was set at α = .05 for all statistical analyses.

[a]For BL-2, $P$ = .032 for PD-1 to PD-6 and $P$ = .047 for PD-4 to PD-6, both significant increases. For BL-3, $P$ = .024 for PD-1 to PD-2 and $P$ = .021 for PD-2 to PD-6, both significant increases. For BL-4, $P$ = .023 for PD-2 to PD-6, a significant increase.

Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

to PD-8 and PD-2 to PD-15, with $P$ = .022 and $P$ = .044, respectively (Figure 4). A similar trend was found for blends BL-2, BL-3, and BL-4 when comparing PD-2 to PD-8 and PD-15, with $P$ = .019 and $P$ = .002, respectively, for BL-2; $P$ = .041 and $P$ = .011, respectively, for BL-3; and $P$ = .034 and $P$ = .011, respectively, for BL-4. (Figure 4).

Upon examining BL-8, statistical significance was reached only when comparing PD-2 to PD-8, with $P$ = .028. No statistical significance was observed when comparing PD-2 to PD-15 (Figure 4). In addition, upon comparing PD-2 to PD-8 for the BL-5 and BL-6 blends, a trend toward an increase in telomerase activity was observed. Finally, statistically significant differences were observed for all 3 blends—BL-5, BL-6, and BL-7—when comparing PD-2 to PD-15, with $P$ = .016, $P$ = .016, and $P$ = .013, respectively (Figure 4). The data also show that telomerase activity was reduced with time in the control cells, which were not treated, when comparing PD-2 to PD-15, with $P$ = .08, but the measure did not reach statistical significance (Figure 4).

Overall, the data suggested that some blends—BL-1, BL-2, BL-3, and BL-4—outperformed the other blends. The blends BL-5, BL-7, and BL-8 were the least efficient at increasing telomerase activity. BL-6 showed a similar telomerase activity to the BL-5, BL-7, and BL-8 (Figure 4).

BL-1, BL-2, BL-3, and BL-4 were compared to treatment with omega-3 FAs, which had previously been shown to increase telomere length, with decreasing $n$-6:$n$-3 omega-3

polyunsaturated fatty acid (PUFA) ratios in a human, double-blind, 4-month, randomized, controlled trial.[26] Because the current study's data indicated significant effects for those first 4 blends by PD-5, the comparison to omega-3 FAs was stopped at PD-6 for evaluation of telomerase activity against PD-1. Overall, the results indicated that the blends outperformed all of the DHA/EPA doses tested.

The current study's data showed that the telomerase activity was reduced with time in the control cells when comparing P-1 with P-6, with $P$ = .044 (Figure 5). A comparison of the effects of various EPA/DHA doses also showed that statistical significance was reached only when comparing P1 with P6 for the omega-3 dose combination of EPA 2.5M/DHA 0.25M, with $P$ = .024 (Figure 5). No statistical significance was observed when comparing other passages, nor were any differences observed with the other dose combinations omega-3 FAs that were tested.

However, a significant increase in telomerase activity was detected with BL-2 when comparing PD-1 to PD-4 and PD-1 to PD-6, with $P$ = .032 and $P$ = .047, respectively (Figure 5). A similar trend was found for BL-3 when comparing P-1 to P-2 and P-2 to P-6, with $P$ = .024 and $P$ = .021, respectively, and for blend BL-4, when comparing P-2 to P-6 with $P$ = .023 (Figure 5).

A trend toward an increase in telomerase activity was observed when comparing PD-1 and the subsequent passages of the BL-1 blend (Figure 5); however, no statistical significance was reached.

**Figure 6.** Effects of BL-1, BL-2, BL-3, and BL-4 on the Cytotoxicity of Topotecan in A549 Cells, With 48 Hours of Treatment
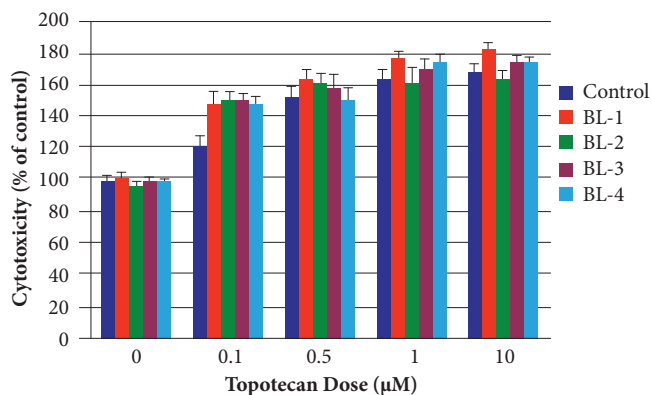


Note: BL-1, BL-2, BL-3 and BL-4 did not affect topotecan's anticancerous activity or toxicity.

**Figure 7.** Effects of BL-1, BL-2, BL-3, and BL-4 on the Antiproliferative Activity of the Chemotherapeutic Agent Topotecan, a Topoisomerase I Inhibitor, on A549 cells, With 48 Hours of Treatment
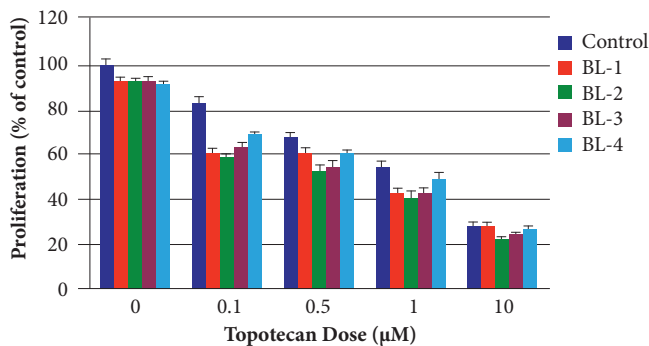


**Effects of the 4 Most Potent Blends on Tumor Cell Proliferation and Survival.** No effects occurred for BL-1, BL-2, BL-3, or BL-4 on viability or proliferation of the A549 cells following 48 hours of treatment (data not shown). In addition, the potential effects of BL-1, BL-2, BL-3, and BL-4 on the cytotoxicity and the antiproliferative activity of the chemotherapeutic agent topotecan were further investigated. The research team found that BL-1, BL-2, BL-3, and BL-4 (Figures 6 and 7) did not affect topotecan's anticancerous activity in A549 cells. Thus, the data indicated that BL-1, BL-2, BL-3, and BL-4 did not appear to have any effects on cell toxicity and/or cell proliferation in vitro in A549 cells.

## DISCUSSION

Substantial experimental evidence has suggested that cell senescence is dependent on cell division numbers and that the total cellular life span is measured by the number of cell generations, not by chronological time.[27,28] Telomere erosion is prevented by the activation of telomerase, a vital enzyme for tissue regeneration. It has been hypothesized that telomerase is suppressed during somatic development; hence, the telomere length shortens with proliferation.[3] Further, investigation of fetal tissues has shown distinct patterns of regulation, with telomerase activity remaining longer in the liver, lung, and spleen.[29]

An aging world population has fueled interest in regenerative remedies that may address declining organ function and provide solutions to maintain fitness. Reducing or slowing cellular degeneration, which is in part caused by telomere erosion, is a key factor in addressing aging at the cellular level. Thus, the current research team explored the potential for natural compounds to slow or reverse the process.

Most of the compounds have previously been described to have some effects on modulating telomerase activity. For instance, broccoli seeds,[30,31] *Chlorella vulgaris* extracts,[32-34] L-carnosine,[35] and vitamin D$_3$[36,37] are all known to affect telomerase activity. In addition, *N*-acetyl-L-cysteine,[38,39] α-lipoic acids,[40-42] green tea extract,[43-45] chia seeds,[46,47] and French wine extract are known to affect cellular aging, both by playing a role in free-radical scavenging and by modulating telomerase. DMAE, known to have antioxidative properties, is used as an antiaging agent,[48,49] whereas rhodiola is used in traditional Chinese medicine.[50-54]

Using a PCR-based assay of telomerase activity, the current study has shown that *N*-acetyl-L-cysteine, L-carnosine, rhodiola PE, vitamin D$_3$, chia seeds, broccoli seeds, and *Astragalus* extract all increased telomerase activity when comparing PD-2 with PD-15 and when comparing those compounds with the control cells, for which the current study observed the expected effect of reduced telomerase activity with time. In addition, DMAE L-bitartrate, *Chlorella* powder, and glycine neither increased nor decreased the telomerase activity, suggesting that they were able to preserve the telomerase activity from PD-2 to PD-15. However, treatment of cells with α-lipoic acid, green tea extract, and French red wine extract appeared to have no beneficial effect on telomerase activity, showing a decrease in telomerase activity with time that was similar to that observed in the control cells.

After investigating the telomerase-modulating effects of all of the single compounds individually, the current research created 8 proprietary blends, with different concentrations and compositions of the compounds based on their performance characteristics during the initial evaluation of telomerase activity. Although it would be feasible simply to pick the best individual compound, other factors, such as synergistic effects, cancer proliferation effects, other adverse events, reliance on a single material source, and future cost considerations, made it clear that it was important to study the combined effects of the compounds.

The current study's data showed that all blends were capable of increasing telomerase activity, when comparing PD-2 to PD-15, with different levels of activation, ranging from 51% to 290% relative to the controls.

The 4 most potent blends—BL-1, BL-2, BL-3, and BL-4—all performed better than TA-65, the positive control, which has been shown to elongate short telomeres and increase the life span of adult and old mice, without any increase in cancer incidence.[15-20,55] Those 4 blends were chosen for further investigation with regard to safety (ie, any effects on cancer proliferation and comparison against human data on other agents promoting telomerase activity).

In a human, double-blind, 4-month, randomized, controlled trial,[24] treatment with PUFA was shown to increase telomere length in relation to the decrease in the $n$-6:$n$-3 PUFA plasma ratios. Human studies to investigate telomerase activity and telomere lengthening in humans need to consider parameters such as sample size and the study's duration, which are not well established at the current time because few such studies have been conducted.

Kiecolt-Glaser et al[24] thus have provided important guidance for the design of future studies because the study demonstrated changes in telomerase activity and telomere lengthening its population of 106 individuals in a 4-month timeframe. In the current study's in vitro assay, the research team compared each of 4 blends against the effects of PUFA combinations. All 4 of the blends outperformed the FA treatments, which supports their further investigation in human studies.

Concerns have been voiced as to the potential cancer-promoting effects of telomerase activators, although to the current research team's knowledge, no experimental evidence exists of such compounds increasing the occurrence of cancer. Although telomerase does not drive the oncogenic process, it is permissive and required for the sustained growth of most advanced cancers. The current study's tumor cell proliferation assay provided no evidence for cancer-promoting effects for its top 4 blends, nor did the blends interfere with the anticancerous effects of the chemotherapeutic topotecan.

Although this study has generated important findings in the field of healthy ageing, we are aware of its limitations and shortcomings. The major limitations herein are listed as follows: (1) the use of a single cell type, (2) the lack of testing the effect of the blend of interest beyond PD-15, and (3) the lack of a companion human study to validate the findings of our in vitro experiments. We believe that these limitations will be addressed and overcome in subsequent work as the Telomerase Plus blend used in this manuscript is currently sold as a nutraceutical and can be used in clinical studies to expand on the existing knowledge of means by which the ingredients exert their beneficial effects.

## CONCLUSIONS

The current study's results have confirmed that many naturally occurring compounds hold the potential to activate telomerase and that certain of those compounds have demonstrated synergistic effects to produce more potent blends. Given the relationship between telomere shortening, aging, and the decline of tissue function, it is reasonable to hypothesize that such telomerase-activating blends may have health-promoting benefits, particularly in relation to aging-associated conditions. Further investigation of such blends in human studies that are designed to evaluate safety and the effects on telomere length are thus warranted.

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