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Ameliorative effects of a beverage containing elastin peptides, *Sophora japonica* flowers and *Haematococcus pluvialis* on skin aging: evidence from cell and human studies

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Abstract

Purpose – Dietary supplements claiming anti-aging benefits often lack robust scientific validation. While elastin peptides (Peptides), *Sophora japonica L*. flower extract with specific content of rutin (Extract) and *Haematococcus pluvialis* powder containing astaxanthin (Powder) have each demonstrated anti-aging properties individually, their combined effects on skin aging remain unexplored. This study aims to evaluate the synergistic anti-aging potential of a beverage combining these ingredients and to validate its efficacy in cellular models and human study.

Design/methodology/approach – Ultraviolet A-induced stress models using human foreskin fibroblasts and human keratinocytes were used to assess collagen, elastin, hyaluronic acid and aquaporin 3 synthesis. A four-week human study with eight participants measured skin elasticity (parameters R_2, R_5 and R_7) and moisture content before and after oral consumption.

Findings – The ingredient ratio 15:8:20 for Peptide, Extract and Powder was optimal, which increased elastin, collagen and hyaluronic acid production by 155.98%, 104.79% and 120.98%, respectively, in cell models. In human study, after four weeks of administration, skin gross elasticity (R_2), net elasticity (R_5) and

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biological elasticity (R_7) increased significantly by 18.70%, 15.50% and 25.39%, respectively. Additionally, skin moisture content increased significantly by 47.80%.

Research limitations/implications – The beverage demonstrated potent anti-skin aging effects, attributed to synergistic antioxidative actions of Peptide, Extract and Powder. This study provides evidence for oral nutraceuticals targeting multiple extracellular matrix components. While the formulation shows application potential for improving skin health, a larger and placebo-controlled trial is warranted to further confirm its efficacy.

Originality/value – The beverage demonstrated potent anti-skin aging effects, attributed to synergistic antioxidative actions of Peptide, Extract and Powder. This study provides evidence for oral nutraceuticals targeting multiple extracellular matrix components. While the formulation shows application potential for improving skin health, a larger and placebo-controlled trial is warranted to further confirm its efficacy.

Keywords Anti-aging, Skin elasticity, Moisture content, Elastin peptides, *Sophora japonica*, *Haematococcus pluvialis*, Astaxanthin

Paper type Research paper

1. Introduction

The skin, the largest human organ, serves a vital role in safeguarding against environmental influences, thermoregulation, sensory perception, immune response and interpersonal interactions (Peate, 2021). As the most important organ constituting the appearance of the human body, aging of the skin and its appendages are the most intuitive signs of human aging. Skin aging is a highly intricate physiological process influenced by both exogenous and endogenous factors (Puizina-Ivić, 2008). Exogenous skin aging is primarily driven by ultraviolet radiation, environmental pollutants, irritants, smoking, high-sugar diets and temperature fluctuations (Misery, 2004). Endogenous skin aging, or intrinsic aging, results from genetically determined cellular degeneration and age-related physiological alterations, including the depletion of the extracellular matrix (ECM) (Geng *et al.*, 2021). Characteristic features of aging skin include decreased elasticity, uneven skin tone, wrinkles, sagging, dryness and chapping (Zouboulis *et al.*, 2019). Chronic ultraviolet (UV) exposure increases epidermal thickness and promotes collagen loss, leading to skin aging. With the rapid economic development and pace of scientific development, people are paying increasing attention to maintaining skin health scientifically and correctly to restore its smoothness and elasticity (Tabor and Blair, 2009).

Collagen is a critical structural component in the human body and the most abundant functional protein, comprising approximately 30% of total protein content (Reilly and Lozano, 2021). It is found in connective tissues such as skin, cartilage, tendons and ligaments. In the skin, collagen is predominantly located in the dermis, forming a major part of the ECM, with type I collagen constituting up to 80% (Patino *et al.*, 2002). The degradation of the ECM, consisting of elastic fibers and collagen, is a key process in skin aging. Elastic fibers are essential for maintaining skin elasticity; their degradation results in skin loosening, wrinkles, plaques and nodules. Elastin, the primary component of elastic fibers (up to 90%), is mainly synthesized by dermal fibroblasts. With aging, elastin becomes more susceptible to hydrolytic enzymes, increasing its degradation during the aging process (Fulop *et al.*, 2012).

Hyaluronic acid (HA) is a non-sulfated glycosaminoglycan, primarily formed through the repeated polymerization of the disaccharides N-acetylglucosamine and D-glucuronic acid. It is widely distributed throughout the human body and, akin to collagen and elastin, serves as a crucial component of the ECM. Approximately 50% of HA is distributed in the dermal ECM, basal layer cells, epidermal spiny cells and epidermal ECM. Aquaporin 3 (AQP3) is a specific protein located on the cell membrane, which has the function of transporting small molecules such as water, glycerol and urea across the membrane. AQP3 is mainly expressed

in keratinocytes and fibroblasts in the skin. Its expression has a spatial hierarchy proportional to the water content in each layer of the skin. AQP3 can transport endogenous triglycerides from glycerol sebaceous glands to the stratum corneum to participate in glycerol metabolism. Therefore, stimulating the synthesis of AQP3 can also increase the water content of the epidermal stratum corneum (Sougrat *et al.*, 2002).

The ECM includes elastin, collagen and HA. They are the main material basis for supporting the structure of the dermis, and a reduction in their quantity and density is the main reason for the collapse of the skin structure, loss of elasticity and the formation of wrinkles. Therefore, promoting the synthesis and uniform distribution of collagen, elastin and HA in the ECM is one of the most effective methods for delaying skin aging and maintaining skin health. Despite growing interest in oral skin beauty supplements, current products face two critical limitations. First, they often require prolonged administration to manifest visible effects, reducing consumer compliance and satisfaction. Second, there are few oral beauty supplements that can specifically increase all three significant components of the skin together (Borumand and Sibilla, 2014; Asserin *et al.*, 2015; Oe *et al.*, 2017). Therefore, the objectives of the present study are, firstly, develop a novel dietary supplement product combining elastin peptides, extract of *Sophora japonica L.* flower and *Haematococcus pluvialis* powder to synergistically target elastin, collagen and HA synthesis. Second is to validate its efficacy in Ultraviolet A (UVA)-induced cellular stress models. Last but not the least, the *in vivo* improvements in skin elasticity and moisture need to be assessed through a four-week human study.

2. Materials and methods

2.1 Materials

The human foreskin fibroblast (HFF-1) and human keratinocyte (HaCaT) cell lines were procured from Sigma, the USA. Phosphate buffered saline (PBS), fetal bovine serum and Dulbecco's Modified Eagle's Medium were also obtained from Sigma, the USA. Epidermal growth factor was sourced from Gibco (Thermo Fisher, the USA), and enzyme-linked immunosorbent assay (ELISA) kits were acquired from CUSABIO and CusAb Co. Pvt. Ltd., the USA. The antibiotic solution (penicillin-streptomycin) was purchased from Himedia, India. The beverage formulation consisted of three main ingredients: elastin peptides, rutin extract and astaxanthin powder. The standardized elastin peptides, derived from bovine ligament and containing 1% of desmosine, were registered under the name Elastech® and sourced from Guangdong HuaPeptides Biotechnology Co. Ltd. (Guangzhou, China). The rutin extract, containing 10% rutin, was extracted from *Sophora japonica L.* flowers and purchased from Jiangyin Tianjiang Pharmaceutical Co., Ltd. (Tianyin, China). The astaxanthin powder, containing 2.5% astaxanthin, was derived from *Haematococcus pluvialis* and obtained from Shanghai Nature Biotechnology Co., Ltd. (Shanghai, China). The beverage was formulated with 75 mg/25 mL of Peptides, 100 mg/25 mL of Extract and 80 mg/25 mL of Powder, mixed and dissolved in 25 mL of deionized water. The pH was adjusted to 3.8 using citric acid monohydrate, and the mixture was then heated in a water bath at 90°C for 1 h. Finally, the beverage was packaged into 25 mL aluminum sachets for experimental use.

2.2 Cell culture

Cell growth was monitored under a microscope to ensure they reached 80%–90% confluence before passage. The old medium was discarded, and the cells were washed one to two times with PBS. Subsequently, 1 mL of trypsin was added, and the culture was incubated at 37°C for digestion. The digestion process was checked every minute under the microscope. When

cells detached or rounded, gentle shaking was used to stop the digestion. Digestion was terminated by adding 2 mL of complete medium, doubling the volume of trypsin. The cells were then collected and centrifuged at 1,000 rpm for 5 min. After discarding the supernatant, the cell pellet was resuspended in fresh medium, thoroughly mixed and inoculated into new culture dishes at a 1:3–4 ratio. The new cultures were incubated in a 5% CO_2 incubator at 37°C, with the medium replaced every two to three days.

2.3 Cell viability test

A hundred microliters of the cell suspension (10,000 cells/well) were seeded into a 96-well plate and incubated in a 5% CO_2 incubator at 37°C for 24 h. Following incubation, the medium was discarded, and 100 μL of various concentrations of test materials were added to each well. The plate was incubated for an additional 24 h. After this period, the test materials were removed, and 100 μL of 10% cell counting kit-8 (CCK-8) reagent, prepared in complete medium, was added to each well. The cells were then incubated for 1–4 h. Absorbance was measured at 450 nm to determine cell viability, calculated using the formula: cell viability (%) = $100 \times [(A_{Material} - A_{Blank})/(A_{Control} - A_{Blank})]$.

2.4 UVA-induced stress model for human foreskin fibroblasts cell

HFF-1 cells were cultured in six-well plates and incubated in a 5% CO_2 incubator at 37°C for 24 h. The cells were then treated with various combinations of the three compounds for 2 h before irradiation. Cells were exposed to 10 J/cm^2 UVA. After irradiation, the medium was removed, and the cells were washed three times with sterile PBS. The cells were then covered with a thin layer of PBS and irradiated again with a dose of 10 J/cm^2 UVA. After irradiation, the cells were incubated in a 5% CO_2 incubator at 37°C for 24 h. Elastin and hydroxyproline concentrations were subsequently measured using ELISA kits from Sigma-Aldrich.

2.5 Air exposure-dryness model for human keratinocytes cell

HA and the expression of AQP3 was measured in HaCaT cells. The cell culture method was similar to that used for the HFF-1 cells. The model group was created by exposing HaCaT cells to an ultra-clean bench, without wind, for 30 min at 25°C. Subsequently, cell viability was reduced to 50%–60%. The mixture was added to the cells and incubated for 24 h. Cells were then digested with preheated trypsin and fixed with 4% paraformaldehyde for 10 min. After fixation, cells were washed with PBS and permeabilized with 0.5% Triton X-100 at room temperature for 5 min. HaCaT cells were blocked with 1% BSA at room temperature for 60 min. Subsequently, 1 μL of AQP3 primary antibody and 0.5 μL of AQP3 secondary antibody were added and incubated at room temperature for 30 min. The mean fluorescence intensity of the cells was measured using a flow cytometer. Additionally, the production of HA by HaCaT cells was quantified using an ELISA kit, based on the optical density values.

2.6 UVA-induced oxidative stress model for human keratinocytes cell

The 96 well plate plates with HaCaT cells were incubated in a 5% CO_2 incubator at 37°C for 24 h. The control group did not receive UVA treatment, whereas the model and sample groups were irradiated with 25 J/cm^2 of UVA for 30 min. Each well was added to 2.5 μM H2DCFH-DA and incubated in a 5% CO_2 37°C for 30 min. The medium was removed, and the cells were washed three times with sterile PBS for three times. A microplate reader was used to read the fluorescence absorbance of the cells at emission 488 nm and excitation 530 nm. The inhibition rate of reactive oxygen species (ROS) was calculated as follows:

$$\text{ROS inhibition rate}(\%) = \left(1 - \frac{T - C_0}{C - C_0}\right) 100\%,$$

where T is the fluorescence intensity of the sample, C is the fluorescence intensity of the model and C_0 is the fluorescence intensity of the control sample.

2.7 The preclinical study design

This preclinical study was conducted at Sirio Pharma Co., Ltd. (Shantou, China) in compliance with the Declaration of Helsinki, according to the protocol approved by the China Ethics Committee of Registering Clinical Trials (protocol number ChiECRCT20210581), and written informed consent was obtained from all the participants. Eight Chinese women were included in the study. This study was conducted between February and April 2021. At the start of the trial, the skin parameters of the subjects were measured to establish the baseline. Subsequently, the subjects orally ingested the tested beverage once daily for four weeks. The selection criteria included individuals aged between 35 and 50 years, who were conscious of having dry and rough skin, with measured skin moisture levels below 60 years. Exclusion criteria comprised no recent treatment with sex hormones within the previous three months and non-pregnancy status. Participants were advised to refrain from excessive eating, drinking, exercise, intense sun exposure, lifestyle alterations and changes in cosmetics. Skin condition was evaluated instrumentally at three time points: baseline before regular ingestion and after two and four weeks of ingestion. Before facial skin evaluation, participants removed their makeup using conventional methods and acclimated for 20 min in a waiting lounge maintained at a constant temperature of 24 ± 2°C and humidity of 55 ± 5%.

2.7.1 Skin moisture measurement. The change in the dielectric constant, assessed via the electrical capacitance method, was used to estimate skin moisture levels at the cheek and canthus. This was accomplished using a CK Multi-probe adaptor (MPA580) with a corneometer probe CM825 (Courage and Khazaka, Cologne, Germany). Three measurements were conducted at each site and averaged, with standard deviations subsequently calculated.

2.7.2 Skin elasticity. Skin elasticity on the cheek of the subjects was evaluated using a Cutometer MPA580 (Courage and Khazaka, Germany) at baseline and two and four weeks after product intake. The test involved suctioning the skin into the probe aperture and releasing it after 2 s, using a noninvasive 2 mm probe pressed onto the skin. Results were derived from three measurements, using Mode 1 with 450 mbar of constant suction for 2 s, followed by 2 s of relaxation time. Parameters R_2, R_5 and R_7, highly correlated with skin elasticity, were assessed. Ua represented the difference between maximum deformation during the initial vacuum period and deformation after 1 s of normal pressure, while Uf indicated the final distension at the end of the first vacuum period. Skin elastic parameter R_2 was calculated as Ua/Uf, reflecting overall skin elasticity. R_5, representing net skin elasticity, was computed as the immediate relaxation within the first 0.1 s after the end of the first vacuum period (Ur) divided by the immediate distension of the skin within the first 0.1 s of the first vacuum period (Ue). Additionally, R_7, indicating the ratio of elastic recovery to total deformation, was determined by Ur/Uf.

2.8 Statistical analysis

Statistical comparison of cellular experiment results, skin moisture and elasticity data at different time points of the subjects was performed using a One-way ANOVA test. Statistical significance was defined as $p < 0.05$. Data analysis was conducted using SPSS Ver. 13.0 (IBM Inc., Armonk, NY, USA). Each value is presented as mean ± standard deviation (SD) of triplicate experiments.

3. Results

3.1 Different combinations of peptides, extract and powder showed no toxicity to both human foreskin fibroblasts and human keratinocyte cells

The Peptides, Extract and Powder were mixed in different composition ratios at the same final concentration (200 μg/mL) to obtain samples containing anti-aging active substances. The composition of the mixture of each sample is shown in Table 1. The effect of the key material components of elastin peptide composition on the cytotoxic viability of HFF-1 and HaCaT were detected using the CCK-8 method. As shown in Figure 1(a), after treatment with different combinations of Peptides, Extracts and Powders, cell viability remained above 80%, indicating that the mixture below these concentrations had no significant cytotoxicity to HFF-1 cells. As shown in Figure 1(b), the combination treatment did not induce significant cytotoxicity in HaCaT cells. Therefore, a formula with different combinations was tested for its anti-aging effects on HFF-1 and HaCaT cells.

3.2 Optimal combination of peptides, extract and powder prevented the UVA-induced decreases in elasin and hydroxyproline in human foreskin fibroblasts cells

The elastin produced by HFF-1 cells is shown in Figure 2(a). In comparison to the model group treated with UVA-induced stress, the sample groups treated with the combination of C

Table 1. The proportion of each ingredient used in the sample formula (200 μg/mL)

Sample no.	Elastin	*Haematococcus pluvialis* powder	*Sophora japonica L.* extract
A	75.0	10.0	30.0
B	75.0	20.0	40.0
C	75.0	27.0	50.0
D	75.0	40.0	100
E	75.0	50.0	150
F	75.0	80.0	200
G	75.0	90.0	210

Source(s): Authors' own creation

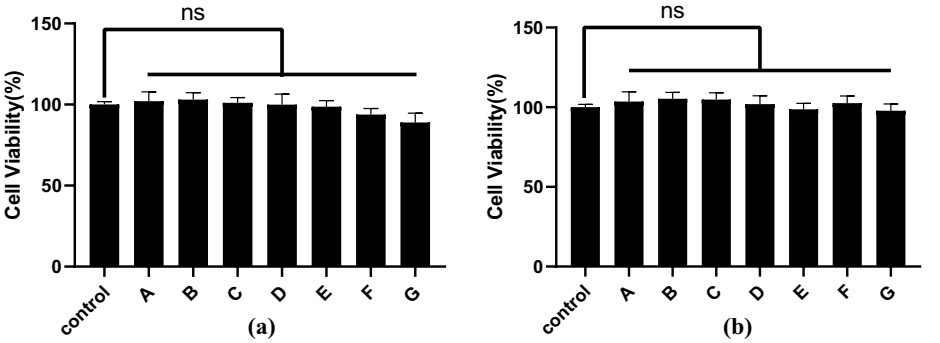

Figure 1. The toxicity of different combination of peptides, extract and powder: (a) cell viability of human foreskin fibroblasts cell; (b) cell viability of human keratinocytes cell
Note(s): Values are mean ± SD of triplicate of experiments
Source: Authors' own creation

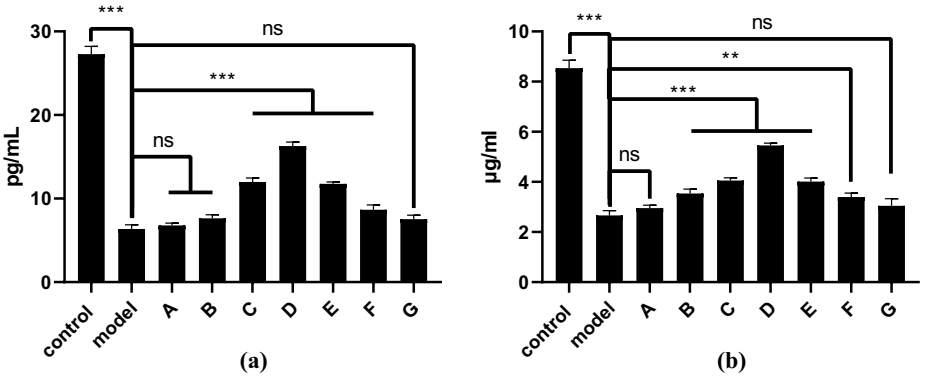

Figure 2. Optimal combination of peptides, extract and powder prevented the UVA-induced decreases in elasin and hydroxyproline in human foreskin fibroblasts cells: (a) the elastin level; and (b) the hydroxyproline level

Note(s): Values are mean ± SD of triplicate of experiments. $^{**}p < 0.01$ and $^{***}p < 0.001$(vs model)

Source: Authors' own creation

and F exhibited significantly higher elastin production. Among the sample groups, group D showed the highest elastin production. The results indicated that when elastin was formulated with the extract of *Sophora japonica L*. and the powder of *Haematococcus pluvialis* at a ratio of 15:20:8, the anti-aging substance had the most prominent effect on promoting elastin production.

Figure 2(b) shows the hydroxyproline content of HFF-1 cells. Compared to the model group treated with UVA-induced stress, the sample groups treated with the combination of B and F exhibited significantly higher production of hydroxyproline. Among the sample groups, group D exhibited the highest production of hydroxyproline. The results indicated that when elastin was formulated with the extract of *Sophora japonica L*. and the powder of *Haematococcus pluvialis* at a ratio of 15:20:8, the anti-aging substance most significantly promoted the production of hydroxyproline.

3.3 Optimal combination of peptides, extract and powder prevented the air exposure-induced decreases in hyaluronic acid and AQP expression in human keratinocytes cells

The generation of HA and the expression of AQP3 were shown in Figures 3(a) and 3(b). The HA was produced in HaCaT cells. Comparing the model group to the sample groups with different combinations of formula, all sample groups exhibited significantly higher production of HA and expression of AQP3. HaCaT cells treated with combination D also showed the highest concentration of HA and the strongest expression of AQP3.

3.4 The optimal combination of the peptides, extract and powder showed synergistic effects in human foreskin fibroblasts cells

To verify the synergistic effect of combination D, elastin and hydroxyproline concentrations were investigated in HFF-1 cells after treatment with Peptides, Extract and Powder individually and in combination with D at the same concentration (200 μg/mL). The results are shown in Figures 4(a) and 4(b). Comparing the model and sample groups, only the samples treated with elastin peptides and combination D showed a promoting

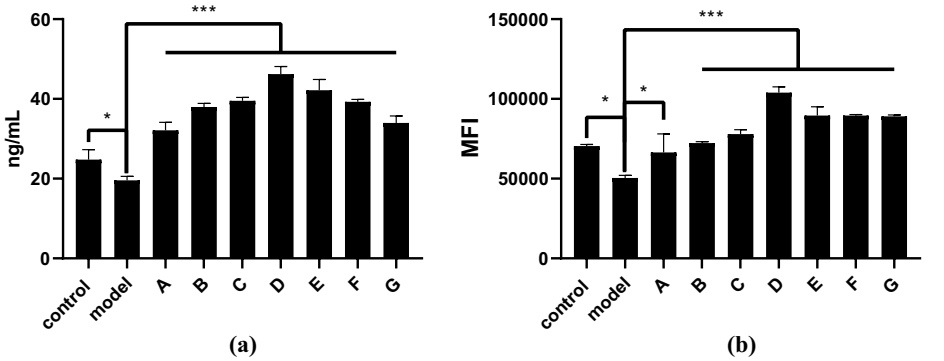

Figure 3. Optimal combination of peptides, extract and powder prevented the air exposure-induced decreases in hyaluronic acid and aquaporins 3 expression in human keratinocytes cells: (a) the hyaluronic acid level; and (b) the expression of aquaporins 3

Note(s): Values are mean ± SD of triplicate of experiments. *$p < 0.05$ and ***$p < 0.001$(vs model)

Source: Authors' own creation

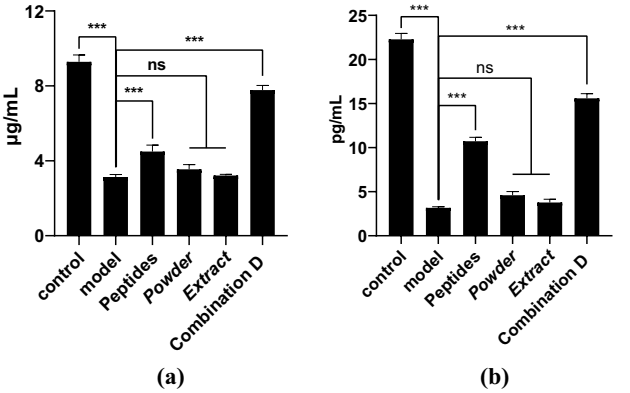

Figure 4. The optimal combination of peptides, extract and powder show synergistic effect in human foreskin fibroblasts cells: (a) the synergistic effect on hydroxyproline level; and (b) the synergistic effect on elastin level

Note(s): Values are mean ± SD of triplicate of experiments. ***$p < 0.001$(vs model)

Source: Authors' own creation

effect on elastin and collagen synthesis. Moreover, the concentrations of elastin and hydroxyproline in the HFF-1 cells treated with combination D were much higher than those in the elastin peptide groups.

3.5 The optimal combination of the peptides, extract and powder showed synergistic effects in human keratinocytes cells

The anti-aging synergistic effect of combination D was also investigated in HaCaT cells by evaluating the concentration of HA and the expression of AQP3 after treatment with elastin

peptides, the powder of *Haematococcus pluvialis*, the extract of *Sophora japonica L*. extract and combination D at the same concentration (200 μg/mL). The results are showed in Figures 5(a) and 5(b). The result of Figure 5(a) indicates that only the samples treated with the extract of *Sophora japonica L*. and the combination D showed promoting effect on HA synthesis. Moreover, compared with the *Sophora japonica L*. group, the concentration of HA in HaCaT cells treated with combination D was much higher. The results in Figure 5(b) demonstrate that all samples promoted the expression of AQP3 compared to the model group, then the group treated with combination D exhibited the highest expression of AQP3. These results indicate that the anti-aging effects of combination D are superior to those of each ingredient alone; however, additional research is required to elucidate the mechanism underlying this synergistic effect.

3.6 The optimal combination of the peptides, extract and powder showed reactive oxygen species inhibitory effect in human keratinocytes cells

The anti-oxidative effects of Peptide, Powder, Extract and combination D at the same final concentration (200 μg/mL) were also investigated in HaCaT cells by evaluating the ROS level induced by UVA-induced stress. The results are shown in Figure 6. The mean fluorescence intensity revealed a significant increase in ROS levels in the model group following UVA irradiation compared to the control group. Among the four samples, except elastin, the powder, extract and combination D exhibited antioxidative effects. The ROS inhibition rates were 87.2%, 70.6% and 78.3% for the Extract, the Powder and the combination D, respectively. There were no significant differences observed in the inhibitory effects of the three groups of samples on ROS production.

3.7 The beverage consumption showed improvement in skin aging in human study

The moisture content in the stratum corneum of the right cheek was measured using a Corneometer CM825. Comparing the mean value of the moisture content of the subjects at baseline to the mean value after two weeks of ingestion of the tested products, the moisture

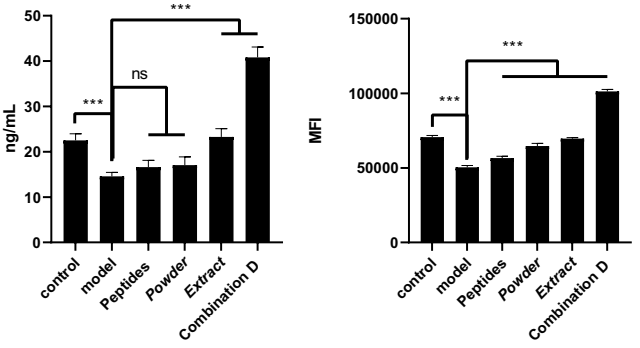

Figure 5. The optimal combination of peptides, extract and powder show synergistic effect in human keratinocytes cells: (a) the synergistic effect on hyaluronic acid level; and (b) the synergistic effect on aquaporins 3 expression

Note(s): Values are mean ± SD of triplicate of experiments. ***$p < 0.001$(vs model)

Source: Authors' own creation

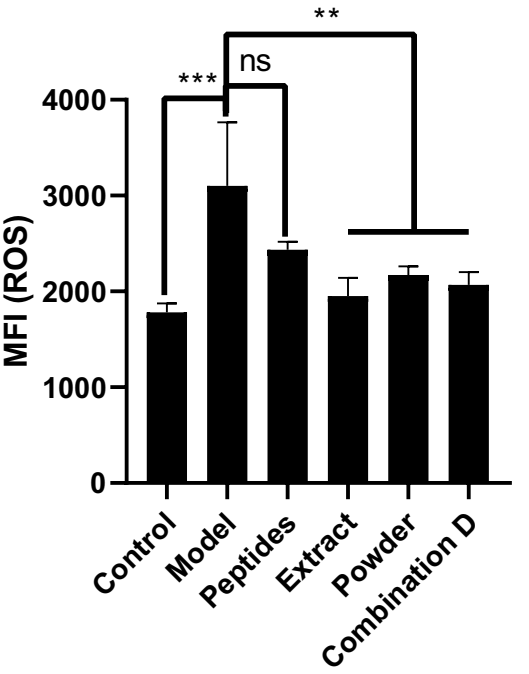

Figure 6. The reactive oxygen species inhibitory effect of peptides, extract, powder and combination D in UVA-induced stress model in human keratinocytes cells
Note(s): Values are mean ± SD of triplicate of experiments. $^{**}p < 0.01$ and $^{***}p < 0.001$ (vs model)
Source: Authors' own creation

content increased significantly by 24.21%. Comparing the baseline with after four weeks of ingestion of the tested products, the moisture content of the subjects increased significantly by 37.62% (Table 2). A clear dose–response relationship was demonstrated by the percentage of moisture content that increased with the consumption period of the tested products.

Table 2. Measured skin parameters and changes for the subjects

			Measured value				
			Before ingestion	Two weeks		Four weeks	
Measured items		Unit	Mean ± SD	Mean ± SD	*p*	Mean ± SD	*p*
Moisture value		A.U.	43.5 ± 11.1	54.7 ± 9.6	0.09	62.2 ± 9.4	0.003
Viscoelasticity	R_2	%	60.0 ± 3.6	66.7 ± 5.0	0.05	71.1 ± 3.9	0.002
	R_5		51.5 ± 6.3	57.2 ± 5.9	0.16	59.2 ± 5.5	0.04
	R_7		35.3 ± 5.5	41.6 ± 5.8	0.09	43.9 ± 5.7	0.002

Note(s): Statistical analysis were performed using a One-way ANOVA test. Statistical significance was defined as $p < 0.05$. Each value is presented as mean ± standard deviation (SD) of triplicate experiments
Source(s): Authors' own creation

As shown in Table 2, the overall skin elasticity R_2 increased significantly by 11.17% after consuming the tested product for two weeks and by 18.70% after four weeks of ingestion. The net skin elasticity R_5 of subjects also increased significantly by 12.27% and 15.50% after ingestion of products for two and four weeks, respectively. Compared to the baseline at Week 0 before consuming the elastin drink, parameter R_7, which describes the overall ability of skin to recover from deformation, also increased significantly by 18.41% and 25.39% after ingestion for two and four weeks, respectively. The results of these elastic parameters of the skin also demonstrated the dose–response relationship of the increased percentage with the ingestion period.

4. Discussion

With chronological and extrinsic aging, human skin physiology undergoes significant changes. Skin aging is typified by a decrease in skin thickness, diminished skin elasticity, degeneration of collagen fibers and the formation of wrinkles (Batisse *et al.*, 2002). Collagen depletion stands out as a primary contributor to skin aging. Research indicates that collagen levels in the body peak around the age of 20 years and progressively decline at an average rate of approximately 1% per year thereafter. Additionally, the capacity of dermal fibroblasts to synthesize collagen I diminishes with age (Shuster *et al.*, 1975). Hydroxyproline is a non-essential amino acid and one of the most important amino acids required for collagen synthesis. It is also a unique amino acid in collagen, accounting for 12%–14% of the total mass of amino acids. The determination of hydroxyproline content can reflect the metabolism of collagen in cells and indicates the amount of collagen produced by cells. With aging, the degradation of HA in the human skin is accelerated, and the expression of HA synthase is significantly downregulated, leading to a significant decrease in HA content in the epidermis (Meyer and Stern, 1994). The water-holding capacity of HA is mainly derived from the fact that its structure contains multiple hydrophilic hydroxyl and carboxyl groups, which can combine with a large number of water molecules to form hydrogen bonds. Therefore, HA is the most important moisturizing substance in the skin, and the degradation of HA content in the epidermis leads to dry skin, damaged skin barrier and increased trans-epidermal water loss rate, all of which are closely related to skin aging (Papakonstantinou *et al.*, 2012). During the aging process, the decline in skin moisture and trans-epidermal water loss is attributed to not only HA degradation but also the downregulation of AQP3. Studies have revealed notable variations in AQP3 protein expression in the skin tissue of individuals across different age brackets. Our findings corroborate that AQP3 expression diminishes with age. The reduced expression of AQP3 results in decreased hydration of the stratum corneum, diminished water and glycerin content in the epidermis and impaired repair of the skin barrier function. Consequently, these factors contribute to dryness, chapping, loss of elasticity and the development of fine lines. In essence, the expression level of aquaporin serves as a pivotal determinant in the natural aging trajectory of the skin and is integral to maintaining its normal structure and function.

In conclusion, the principal physiological alteration observed in skin aging is the metabolism of the dermal ECM. With aging, the interaction between the ECM and fibroblasts is disrupted, as fibroblasts synthesize fewer ECM proteins and more matrix-degrading metalloproteinases (MMP) (Cole *et al.*, 2018). Thus, strategies aimed at delaying degradation or promoting the production of significant ECM components represent the most prominent approach to combat skin aging.

To date, only a limited number of clinical studies have explored the effects of oral elastin ingestion on skin parameters. Elastin hydrolysate has been shown to enhance cell proliferation and elastin synthesis in human fibroblasts. The intrinsic mechanism of anti-skin aging effect of elastin peptides has just been explored by a recent study which showed that the intervention of elastin peptides improved gene expression of TGF/*β*/Smad pathway-related genes such as *P4ha2*, *Admst2*, *Bmp1* and *Lox*, while the expression of protein *Ap-1*

was downregulated and lead to the inhibition of MMP-1. Therefore, the synthesis and degradation of collagen is balanced to maintain dermal integrity and reduce the development of skin wrinkles (Liu *et al.*, 2025). In a double-blind, placebo-controlled study investigating the impact of oral administration of elastin hydrolysate on skin condition, 20 healthy subjects were equally divided into elastin and placebo groups. Following four weeks of administration, improvements in skin elasticity and reductions in the number and volume of wrinkles were observed. Importantly, the enhancements in the elastin group surpassed those in the placebo group (Shiratsuchi *et al.*, 2016). Considering the dosage used in clinical studies, 75 mg of elastin hydrolysate was deemed effective when combined with the formula.

ROS are extremely unstable and can easily interact with proteins, lipids and DNAthrough chain reactions, ultimately leading to peroxidation of proteins, lipids and DNA damage. Therefore, oxidative stress is the primary cause of skin ageing. Astaxanthin has been added to the formula as a strong antioxidant. Because it has a long chain of conjugated olefins, astaxanthin can stop the chain reaction by neutralizing singlet oxygen and scavenging free radicals to play an antioxidant role. The unique molecular structure of astaxanthin enables it to penetrate the cell membrane and inhibit lipid peroxidation. Therefore, astaxanthin has stronger antioxidant activity than lutein, lycopene, α-carotene or β-carotene (Satoh, 2016). Astaxanthin has also been shown to have anti-UV effects. The levels of inflammatory factors and MMP-1 in HaCaT irradiated with UVA were significantly increased. Another research demonstrated that UV induce skin aging by the stimulation of COX2, IL-8, GM-CSF and TGase1 through NFκB pathway. On the other hand, astaxanthin can prevent skin aging by inhibiting MSK1 phosphorylation, therefore suppressing the p38/MSK1/NFκBp65Ser276 axis in keratinocytes to reduce inflammation and abnormal keratinization (Imokawa, 2019). The experimental group supplemented with astaxanthin in the cell culture medium showed significantly reduced levels of related inflammatory factors and MMP-1 compared with the control group, which explored the mechanism of astaxanthin in preventing UVA-induced skin aging *in vivo* (Tominaga *et al.*, 2017). Several studies have demonstrated that oral ingestion of astaxanthin can ameliorate various skin conditions, including reducing the volume and number of wrinkles, enhancing skin elasticity, improving the moisture content of the stratum corneum and reducing transepidermal water loss (Tominaga *et al.*, 2012; Yamashita, 2005). The typical astaxanthin dosage used in these clinical studies ranged from approximately 4 to 12 mg per day.

The extract of *Sophora japonica L*. flowers was added to the formula as a potent antioxidant. As the extract of *Sophora japonica L*. flowers contains many polyphenols, polysaccharides and flavonoids, such as rutin, quercetin and genistein, it also exhibits strong antioxidative ability. The extract of *Sophora japonica L*. flowers has been reported to have high DPPH scavenging activity, hydroxyl radical scavenging activity and superoxide radical scavenging activity, and its antioxidative ability is comparable to that of the positive control ascorbic acid (Lingwen *et al.*, 2013). Rutin, the major compound in the extract of *Sophora japonica L*. flowers, has also been reported to exhibit anti-aging activity. The anti-aging potentials of rutin was revealed by a study tested the inhibition of collagenase, elastase and hyaluronidase of rutin and the IC_{50} were 104.70 μg/mL, 46.88 μg/mL and 114.07 μg/mL, respectively (Girsang *et al.*, 2020). Furthermore, another study showed that rutin increased the mRNA expression of collagen type I alpha 1(COL1A1) and decreased the mRNA expression of MMP-1 in human dermal fibroblasts (Choi *et al.*, 2016). Another study demonstrated that rutin protect human skin fibroblast from UVA-induce aging by activating the Nrf2 signaling pathway, which reduce oxidative stress through the increasing of glutathione, suppression of cell apoptosis by elevation of Bcl2/Bax ratio and the preservation of mitochondrial functions (Tabolacci *et al.*, 2023).

Although our study provides anti-skin aging potential of the beverage containing elastin peptides, there are several limitations. First, we observed some significant improvements in skin health parameters of the subjects, the beneficial effects were from the comparison between the readouts before and after the intervention. Second, the sample size of the study is relatively small and might reduce the statistic power of the analysis. Third, the recruited subjects are all women in the age group of 35–50 years old. Last but not the least, we acknowledge that the *in vitro* skin cell models cannot fully replicate the complexities of human digestion and systemic absorption. These limitations may hinder its extrapolation. A clinical study with a control group on placebo formula should be the next approach. In future work, we also plan to conduct simulated gastrointestinal digestion assays to quantify the residual bioactivity of the ingredients following digestion and test the metabolites on skin cell models.

5. Conclusion

In this study, the anti-skin aging effects of a mixture comprising elastin peptides, astaxanthin and an extract of *Sophora japonica L.* flowers were evaluated using HFF-1 and HaCaT cell models. Various combinations of these ingredients were tested to determine the optimal formula. The mixture containing elastin peptides, astaxanthin and *Sophora japonica* extract at a ratio of 15:8:20 exhibited the most significant effects in promoting collagen, elastin and HA synthesis, as well as AQP3 expression, in both HFF-1 and HaCaT cells.

Specifically, the Combination D – containing the optimal ratio – enhanced elastin, collagen and HA production by 155.98%, 104.79% and 120.98%, respectively. Following confirmation of the optimal formula through cell-based assays, food samples prepared using this formulation were used in a preclinical trial to evaluate anti-skin aging effects in healthy human subjects. After four weeks of consumption, significant improvements were observed in skin elasticity parameters R_2, R_5 and R_7, which increased by 18.70%, 15.50% and 25.39%, respectively. Additionally, skin moisture content increased by 47.80% compared to baseline.

In conclusion, this study addresses a critical gap in oral skin health interventions by demonstrating that a single formulation can simultaneously enhance elastin, collagen and HA synthesis – key components responsible for maintaining skin elasticity and hydration. The rapid onset of observable benefits (within two to four weeks) positions this beverage as a promising nutraceutical for combating skin aging, with potential applications in cosmeceuticals and functional foods. This work lays a foundational framework for the development of multi-target oral supplements aimed at addressing the complex mechanisms of skin aging. Future research should include larger-scale, double-blind, placebo-controlled clinical trials across diverse populations to further validate long-term efficacy and safety.

Author contributions

Data curation, formal analysis, writing – original draft preparation, D.Y.; methodology, J.Z., resources, S.H., J.S.; investigation, J.Z., J.S., D.Y.; writing – review and editing, J.L.; Conceptualization, supervision, P.C. All authors have read and agreed to the published version of the manuscript.

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