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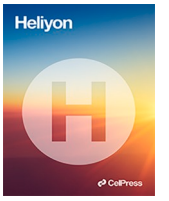

Research article

Synergistic effect of *Panax ginseng, Polygonatum cyrtonema, Epiphyllum oxypetalum, Nelumbo nucifera and Osmanthus fragrans* extracts on skin aging regulation. From *in silico* predictions to *in vitro* outcome

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ABSTRACT

Intrinsic and extrinsic aging affect the health of human skin. Extracellular matrix protein degradation, DNA damage and oxidative stress are known to disturb skin architecture and skin homeostasis leading to skin aging. Traditional Chinese Medicine (TCM) delivers a large amount of knowledge regarding the phytotherapeutic power of diverse plants. *Panax ginseng*, *Polygonatum cyrtonema*, *Epiphyllum oxypetalum*, *Nelumbo nucifera* and *Osmanthus fragrans* are five plants used in TCM for their protective effect. In this study, several combinations of these TCM plants were explored: first, an *in silico* analysis was performed to predict their potential to target biological activities in the skin and then, some predictions were verified with *in vitro* studies to underline the synergistic effect of plant extracts. The results showed a stronger anti-aging activity for the combination with the five plants compared to the combination with *Panax ginseng*, *Polygonatum cyrtonema*, *Epiphyllum oxypetalum* and, compared to *Panax ginseng* alone.

1. Introduction

Skin aging is the consequence of both intrinsic and extrinsic factors leading to the degradation of skin integrity and skin physiological functions [1,2]. The skin is daily exposed to extrinsic factors responsible for aging such as ultraviolet irradiation, pollution, mechanical and bacterial stresses. In parallel, genetic, ethnic variabilities and hormonal changes lead to intrinsic aging with functional decrease of internal machinery.

Nine hallmarks of aging were described by Lopez and collaborators: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, dysregulated nutrient-sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, altered intercellular communication; and completed by Schmauck- Medina and collaborators with other hallmarks such as compromised autophagy, microbiome disturbance, altered mechanical properties, splicing dysregulation, and inflammation [2,3]. These multiple hallmarks characterize the aging phenotype and can be studied using specific markers. Indeed senescence-associated

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beta-galactosidase (SA-beta-gal) activity is a main marker to study the switch mechanism used by cells to enter in senescence [4]. The γH2AX marker, which is a sensor of DNA double-strand breaks [5], allows to follow genomic instability. Moreover, as skin ages, the protein synthesis and the mRNA expression of ECM markers, as collagen I and collagen III, decrease in senescent cells [6,7].

Global well-being is essential in our life. The first sign of this state is the health and the beauty of our skin. To improve the appearance and the condition of our skin, it is necessary to fight against the characteristics of the aging phenotype. The Traditional Chinese Medicine (TCM) is a tradition belonging to a 2200-year-old ancient civilization and includes various forms of therapy such as acupuncture, massage, exercise, dietary therapy, and herbal medicine. This ancestral treasure is at the origin of the derived use of plants related to Chinese medicine in cosmetic applications. Indeed, the use of plant extracts plays a key role in skin anti-aging and the mix of different plant extracts is an interesting approach to pool their different anti-aging potential to target cosmetic claims.

Panax ginseng, one of the most popular TCM plants, is known for its beneficial effects such as anti-inflammatory, improvement of lipid and glucose metabolism, antioxidation, inhibition of cell apoptosis [8]. Other effects of *Panax ginseng* are also described in skin such as the decrease of hyperpigmentation [9], the protection against photodamage [10] and the repair of skin barrier [11]. Several of these anti-aging effects are due to the main bioactive compounds of *Panax ginseng*: the ginsenosides (also named panaxosides). Among the thirty ginsenosides of *Panax ginseng*, Rb1, Rb2, Rc, Re and Rg1 are the most represented (70–80% of total ginsenosides in fresh *Ginseng*).

Polygonatum cyrtonema is an important plant in TCM through its different abilities to fight against age-related disorders induced by inflammation and oxidation [12].

Epiphyllum oxypetalum, also called "Queen of the Night" and "Night-blooming cactus" is a less well known traditional medicinal plant, described to improve wound healing. Other pharmacological activities were also identified such as antioxidant, anti-inflammatory, and anti-microbial effects [13,14].

Nelumbo nucifera, also called Sacred Lotus, is an important and potent plant in traditional medicines revered in Asia as a divine symbol. Recent molecular research has shown that lotus could be a "living fossil" that already existed more than 138 million years ago. Under favorable circumstances, the lotus seeds may remain viable for hundreds of years [15]; with the oldest recorded lotus germination being from that of seeds 1300 years old recovered from a dry lakebed in northeastern China. This survival potential could confer a strong disposition to *Nelumbo nucifera* to fight against aging process through its secondary metabolites. *Nelumbo nucifera* is also associated with good health and healing. It was described as targeting markers associated with visible signs of skin aging, including skin hydration, barrier function, skin wrinkles, lipolysis, and skin pigmentation [16].

Finally, *Osmanthus fragrans* has been used as folk medicine for thousands of years. The extracts of *Osmanthus fragrans* flowers were reported to have various bioactivities including free radical scavenging and anti-inflammation [17]. Skin whitening properties were also reported in cosmetic use [18].

Research related to the synergy of different plant extracts was rarely conducted and described in the literature. In this study, we evaluated different combinations of plant extracts (*Panax ginseng* root extract, *Polygonatum cyrtonema* rhizome/root extract, *Epiphyllum oxypetalum* flower extract, *Nelumbo nucifera* extract and Osmanthus fragrans flower extract). First, an *in silico* approach was conducted using the phytochemical composition and some validated proteinic targets of each extract to predict potential biological activities. In a second time, *in silico* predictions were assessed through *in vitro* studies. Thus, the anti-aging potential of plant extract combinations were evidenced focusing on different markers with significant role in maintenance of skin structure and youth and related to senescence and DNA damage.

2. Materials and methods

2.1. Plant extracts

Panax ginseng root extract, *Polygonatum cyrtonema* rhizome/root extract and, *Epiphyllum oxypetalum* flower extract were prepared by Shanghai Jahwa United Co., Ltd. They were extracted following the preparation procedure reported in Ref. [19].

The *Nelumbo nucifera* extract was obtained using Ashland proprietary Zeta Fraction technology free solvent process described in Ref. [20].

Table 1
Phytochemical compounds of interest in TCM extracts.

Plant extracts	Phytochemical compounds
Epiphyllum oxypetalum flower extract	quercetin 3-O -β- D-galactoside
	isorhamnetin
Panax ginseng root extract	panaxoside Rg1
	panaxoside Rb1
	panaxoside Re
Polygonatum cyrtonema rhizome/root extract	kaempferol
	liriodendrin
	myricetin
Osmanthus fragrans flower extract	caffeic acid
	p-coumaric acid
	syringic acid

Osmanthus fragrans extract was developed using Ashland proprietary PSR™ technology with some adjustments described in Ref. [21].

2.2. Phytochemical compounds of interest

Phytochemical compounds were identified from previous analytical study on 4 TCM extracts (Table 1). The main phytochemical compounds of *Epiphyllum oxypetalum* flower extract identified using UV spectrometer, High Performance Liquid Chromatography coupled with Mass spectrometer (HPLC-MS) and Nuclear Magnetic Resonance (NMR), are quercetin 3-O -β- D-galactoside and isorhamnetin (Table 1). *Panax ginseng* root extract contains panaxoside Rg1, Rb1 and Re, identified by HPLC coupled with UV and fluorescence detector (Table 1). Kaempferol, liriodendrin and myricetin are the main compounds of *Polygonatum cyrtonema* rhizome/root extract, determined by UV spectrometer, IR spectrometer, Fast Atom Bombardment Mass Spectrometry and NMR (Table 1). *Osmanthus fragrans* flower extract contains caffeic, *p*-coumaric acid and syringic acid identified by HPLC-MS (Table 1). From these data, the simplified molecular-input line-entry system (SMILES) files were retrieved using Pubchem database (PubChem (nih.gov)) for each compound of each extract. The quercetin and hyperoside corresponding to the associated sugar of quercetin was used as SMILES files for the quercetin 3-*O*-β-D-galactoside, D-galactoside.

2.3. Plant extracts combinations

Different combinations of these extracts were used for *in silico* and *in vitro* analysis: (i) 0.5% *Panax ginseng* root extract only (1 TCM), (ii) the combination of 3 TCM plant extracts: 0.167% *Panax ginseng* root extract, 0.166% *Polygonatum cyrtonema* rhizome/root extract and 0.167% *Epiphyllum oxypetalum* flower extract (3 TCM), (iii) the combination of 5 TCM plant extracts: 0.1% *Panax ginseng root extract*, 0.1% *Polygonatum cyrtonema* rhizome/root extract, 0.1% *Epiphyllum oxypetalum* flower extract, 0.1% *Osmanthus fragrans* flower extract and 0.1% *Nelumbo nucifera* extract (5 TCM) (Table 2).

2.4. Validated targets

Some of the TCM plant extracts have been tested individually in previous biological *in vitro* analysis. *Epiphyllum oxypetalum* flower extract had 7 validated targets. *Polygonatum cyrtonema* rhizome/root extract had 6 validated targets. *Osmanthus fragrans* flower extract and *Nelumbo nucifera* extract modulated 2 and 15 targets respectively [16,22] (Table 3).

2.5. Target predictions

Target predictions were performed by the input of SMILES files of each phytocompounds on a published database SwissTargetPrediction [23] and processed based on the target/ligand similarity principle. Then, predicted targets corresponding to each TCM plant extract were listed by Uniprot ID, pooled and duplicates were discarded.

2.6. Gene enrichment study

Target enrichment was performed using Database for Annotation, Visualization, and Integrated Discovery (DAVID) [24,25]. The gene enrichment was conducted in 3 parts: (i) on predicted target genes of 1 TCM, (ii) on predicted target genes and additional *in vitro* validated targets for 3 TCM, (iii) on predicted target genes and additional *in vitro* validated targets for 5 TCM. The DAVID application provided functional annotation to understand the biological significance behind the established lists of genes, based on a modified Fisher's exact test (EASE score) [25]. The level threshold of the p-Value for selection of functional annotations was set to 0.05. The DAVID database gives enriched biological themes, particularly Gene Ontology (GO) terms, terms associated with KEGG pathway, WikiPathways and Reactome data sources. Then, a comparison of annotation terms was performed between the result for 1 TCM, 3 TCM and 5 TCM combinations.

2.7. Cell culture

For collagen I and III detections, normal human dermal fibroblasts (NHDF) (Guangdong Boxi Biotechnology Co., Ltd,

Table 2
Composition of TCM combinations.

TCM plant extracts:	1 TCM	3 TCM	5 TCM
Panax ginseng root extract	0.5%	0.167%	0.1%
Polygonatum cyrtonema rhizome/root extract	/	0.166%	0.1%
Epiphyllum oxypetalum flower extract	/	0.167%	0.1%
Osmanthus fragrans flower extract	/	/	0.1%
Nelumbo nucifera extract	/	/	0.1%
Total	0.5%	0.5%	0.5%

Table 3
in vitro validated targets for TCM plant extracts.

TCM plant extracts:	Uniprot ID:
Epiphyllum oxypetalum	P02452, P02461,P02462, Q02388, P15502, P07585, P13611
Polygonatum cyrtonema	P05231, P10145, P13500, P03956, P16104, P38936
Nelumbo nucifera	P08246, Q92839, Q92819, O00219, P20930, Q92482, P02452, P35354, P10145, P08254, P14679, P17643, P40126, Q05469, Q99685
Osmanthus fragrans	P20930, P14679

Lot#Fb19052002) were seeded at 2×10^5 cells in 6-well plates in low-sugar DMEM culture medium (Gibco) and incubated overnight at 37 °C in a humidified atmosphere containing 5% of CO_2. At 40%~60% confluence, cells were treated with the different TCM plant combinations (Table 2) or with 100 ng/ml TGFβ1 as positive control for 24h. After 24h, each well was washed twice with PBS 1X. Three independent experiments were performed.

For evaluation of SA-β-galactosidase activity and γH2AX detection, NHDF were seeded at 1×10^5 cells in 6-well plates in low-sugar DMEM culture medium and incubated overnight at 37 °C in a humidified atmosphere containing 5% of CO_2. To induce DNA damage and senescence, cells were stressed with H_20_2 and submitted to replicative senescence. Indeed, cells were treated with 400 μM H_20_2 in serum free medium for 2h and washed three times with PBS 1X. In the next step, cells were treated with the different TCM combinations (Table 2) and with 100 ng/ml TGFβ1 for 24h. After 24h, the cells were washed again with PBS 1X three times. These two steps were repeated three times. After the last treatments with TCM combinations and TFGβ1, the cells were sub-cultured for 5 generations.

2.8. Detection of collagen I and collagen III mRNA by Real-Time PCR

Total RNAs were extracted by using RNA iso Plus (Takara). cDNA was synthesized from the isolated RNAs using reverse transcription kit (Takara). Quantitative PCR was performed using COL1A1 and COL3A1 specific primers (SYBR Green). The relative quantification of target expression was determined by using the comparative Ct method. In the comparative Ct method, the Ct values obtained from two different samples are directly normalized to a housekeeping gene (Actin) and then compared. Finally, the results were expressed as mean standard deviation (SD) of 3 independent experiments.

2.9. Detection of SA-β-galactosidase activity

For SA-β-galactosidase activity detection, the previously obtained H_2O_2-stressed senescent cells were seeded into 6-well plates at 80%–90% confluence and incubated at 37 °C in a humidified atmosphere containing 5% of CO_2 for 24h. Cells were washed once with PBS 1X, and SA-β-galactosidase activity was detected using the "cell aging β-Galactosidase staining" kit (Biyuntian) as described in the supplier protocol. Pictures were acquired with an optical microscope (Olympus CKX41) and blue-stained cells were counted in ten fields. The results were expressed as the percentage of positive cells.

2.10. Western blotting for detection of γH2AX

To investigate the level of senescence-related protein γH2AX, Western Blotting analysis was performed. Cells were lysed in RIPA lysis buffer, and proteins were separated using SDS-12% polyacrylamide gel electrophoresis (SDS-PAGE) and blotted onto Immobilon P membrane (Millipore). Then, γH2AX (Abcam) and β-actin antibodies were used at a 1:200 and 1:10000 dilution respectively and secondary goat anti-rabbit IgG HRP (Bio-Rad) was used at a 1:2000 and 1:10000 dilution. The chemiluminescence was detected with Tanon 5200 Multi Chemiluminescence Image System. Quantification of protein bands was established by Band-Scan software (PROZYME, San Leandro, California).

2.11. Statistics

Data was graphed using GraphPad Prism (PraphPad Software), expressed as Mean ± SD. The student *t*-test statistical analysis was

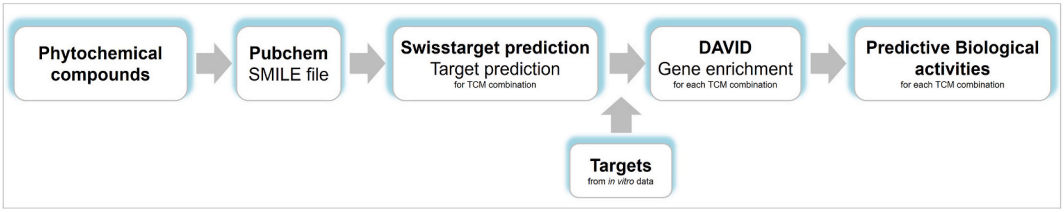

Fig. 1. *In silico* analysis workflow to determine predicted targets and potential predictive biological activities impacted by the 3 combinations of plant extracts.

used for comparison between groups, and the statistical analysis was double-tailed with the Dunnett's test. It was considered $p \leq 0.05$ as significant, $p \leq 0.01$ as very significant and $p \leq 0.005$ as highly significant.

3. Results

3.1. Target prediction

The *in silico* analysis was performed through the modified workflow from Wang article [26] (Fig. 1). The SwissTarget prediction based on phytochemical compounds allowed to identify 120 potential targets for *Epiphyllum oxypetalum* flower extract, 33 targets for *Panax ginseng* root extract, 120 for *Polygonatum cyrtonema* rhizome/root extract and 72 for *Osmanthus fragrans* flower extract. The compilation of predictive targets with the additional *in vitro* validated targets (Table 1) for each TCM plant combination allowed to establish a list of 225 potential targets for the 5 TCM, 173 potential targets for the 3 TCM and 33 potential targets for the 1 TCM (Table 2_supplementary material).

3.2. Gene enrichment

The lists of genes identified for each TCM combination were analyzed with DAVID to determine the terms associated with the most relevant potential biological activities. This study highlighted anti-aging and anti-senescence as the main predictive potential biological activities for each combination (supplementary data_Table 1), with the better significance for the 5 TCM. Indeed, the following terms were identified: "cellular senescence", "FoxO signaling pathway", "longevity regulating pathway", "aging", "mTOR signaling pathway". Other terms associated with hallmarks of aging were also listed. Thus, the autophagy regulation, mitochondrial homeostasis, proteolysis, ubiquitination, and proteasome functions were characterized as predictive potential biological activities targeted by the combination of plant extracts with the better significance for the 5 TCM (pValue 5 TCM < pValue 3 TCM < pValue 1 TCM). The anti-glycation predictive potential was also revealed with the potential regulation of the AGE/RAGE pathway impacting skin aging [27].

In addition, the 5 TCM and 3 TCM blends could potentially regulate the senescence-associated secretory phenotype (SASP) involving protein secretion induced during senescence process including inflammatory cytokines, growth factors and matrix remodeling factors [28]. No prediction suggested a role of *Panax ginseng* root extract alone in that process.

The analysis highlighted other potential biological activities related to anti-aging for the 5 TCM and 3 TCM such as "telomere protection", "Protein folding integrity/homeostasis", "Chromosome organization" and "DNA damage response" mainly with "Base excision repair" process, with a better significance for the 5 TCM.

The predictive impact on histone modification was identified only for the 5 TCM combination. Interestingly, the term "histone demethylase activity" could correspond to the potential regulation of H3K9 and H3K36 methylation involved in the epigenetic regulation of SASP [29].

Moreover, other potential biological activities have been identified for all combinations: epidermal homeostasis, cell to cell adhesion and junctions. The establishment of skin barrier and the water transport are predictive characteristics identified for the 5 TCM combination only. The *in silico* analysis showed the potential impact of each combination on the dermal regeneration, on the extracellular matrix and hyaluronan homeostasis with a better significance potential for the 5 TCM combination (pValue 5 TCM <

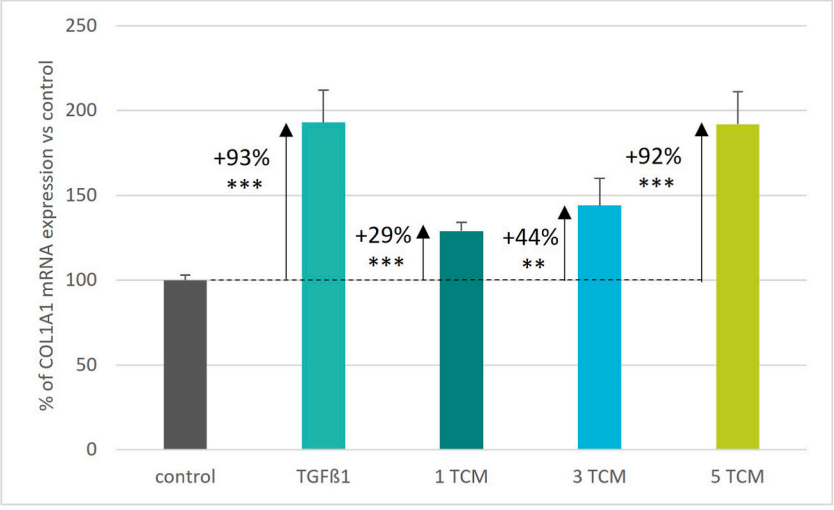

Fig. 2. Evaluation of COL1A1 mRNA expression in fibroblasts treated with TCM combinations for 24h using Real-Time PCR. Statistical analysis was carried out with the Student's *t*-test compared to the control condition. (n = 3, **: very significant, ***: highly significant).

pValue 3 TCM < pValue 1 TCM). The 5 TCM combination could potentially target the metabolic process of global skin glycosaminoglycans known as chondroitin sulfate, dermatan sulfate, keratan sulfate, heparan sulfate, heparin, and hyaluronic acid [30]. Other potential activities were identified and attributed to all TCM combinations such as regulation of cellular proliferation and differentiation, immune response and anti-inflammation, and stress response specifically on the oxidative stress response. The response to xenobiotics or pollutants was highlighted through the potential regulation of the aryl hydrocarbon receptor pathway known to be involved in this response [31].

The *in silico* analysis allowed to predict strong ability to TCM combinations to play on anti-aging and thus orient the following *in vitro* studies on dermal proteins, senescence and DNA damage.

Other domains of the skin biology were also predicted such as adipocyte homeostasis, lipid metabolism, vitamins homeostasis and metabolism, skin tone modulation, melatonin metabolism, HSP90 and cannabinoid pathways with a better significance for the 5 TCM combination. Finally, a predictive regulation of the hair development was determined for the 5 TCM combination only.

3.3. Collagen mRNA expressions

The COL1A1 mRNA expression was significantly increased in cells treated with 1 TCM, 3 TCM and 5 TCM combinations by 29%, 44%, 92% respectively compared to control condition (Fig. 2), meaning that the 5 TCM application has a better efficacy than the 3 TCM application which has a better efficacy than the 1 TCM application. The positive control, TGFβ1, induced an increase of COL1A1 mRNA expression equivalent to the 5 TCM condition (+93%).

The COL3A1 mRNA expression was significatively increased in cells treated with 1 TCM, 3 TCM and 5 TCM combinations by 55%, 72%, 113%, respectively compared to control condition (Fig. 3). In line with results obtained for COL1A1, the 5 TCM application has a better efficacy than the 3 TCM application which has a better efficacy than the 1 TCM application on COL3A1 expression. The positive control, TGFβ1, induced an increase of COL3A1 mRNA expression equivalent to the 3 TCM condition (+87%).

3.4. SA-β-galactosidase activity

Results showed that H_2O_2 stress increased by 262% the number of positive senescent cells compared to the control condition, moving from 5.97% of SA-β-gal positive cells in the control to 21.6% in the H_2O_2 stress condition (Fig. 4 (a)(b)). The application of 1 TCM decreased by 50% the SA-β-gal positive cells, while the application of the 3 TCM decreased by 90% and the application of the 5 TCM decreased by 96% compared to stress condition. Thus, the 5 TCM exhibited again a better efficacy than the 3 TCM which exhibited a better efficacy than the 1 TCM. Interestingly, the TGFβ1 condition had an equivalent efficacy than the 3 TCM by reducing the SA-β-gal positive cells (−88%).

3.5. γH2AX sensor of DNA damage increasing with aging

To further investigate the anti-aging properties of the plant extract combinations, the γH2AX detection in H_2O_2-senescent cells was performed. A highly significant increase of γH2AX protein was observed in H_2O_2-senescent cells compared to the control condition (Fig. 5(a)(b)). The application of 1 TCM, 3 TCM and 5 TCM combinations induced a decrease of γH2AX protein level in H_2O_2-senescent cells (−87%, −32%, −94% respectively). The 5 TCM combination allowed to retrieve the basal level of γH2AX level. Again, TGFβ1 had

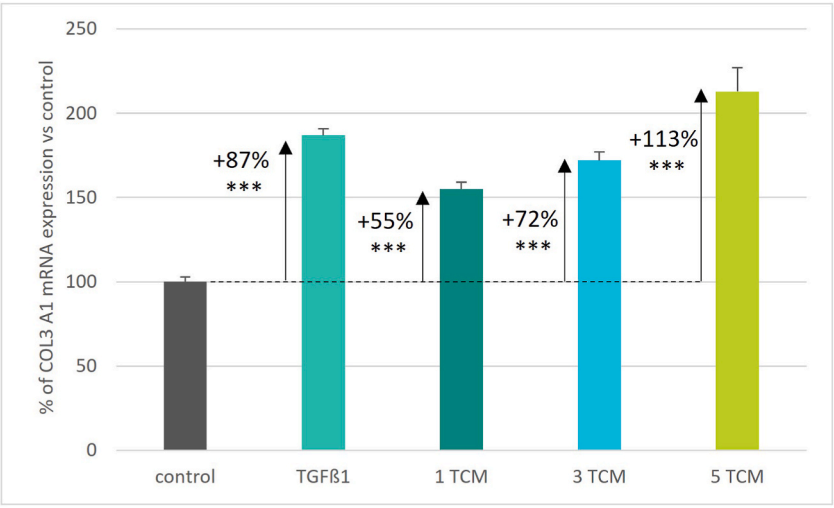

Fig. 3. Evaluation of COL3A1 mRNA expression in fibroblasts treated by TCM combinations for 24h using Real-Time PCR. Statistical analysis was carried out with the Student's t-test compared with the control condition. (n = 3, ***: highly significant).

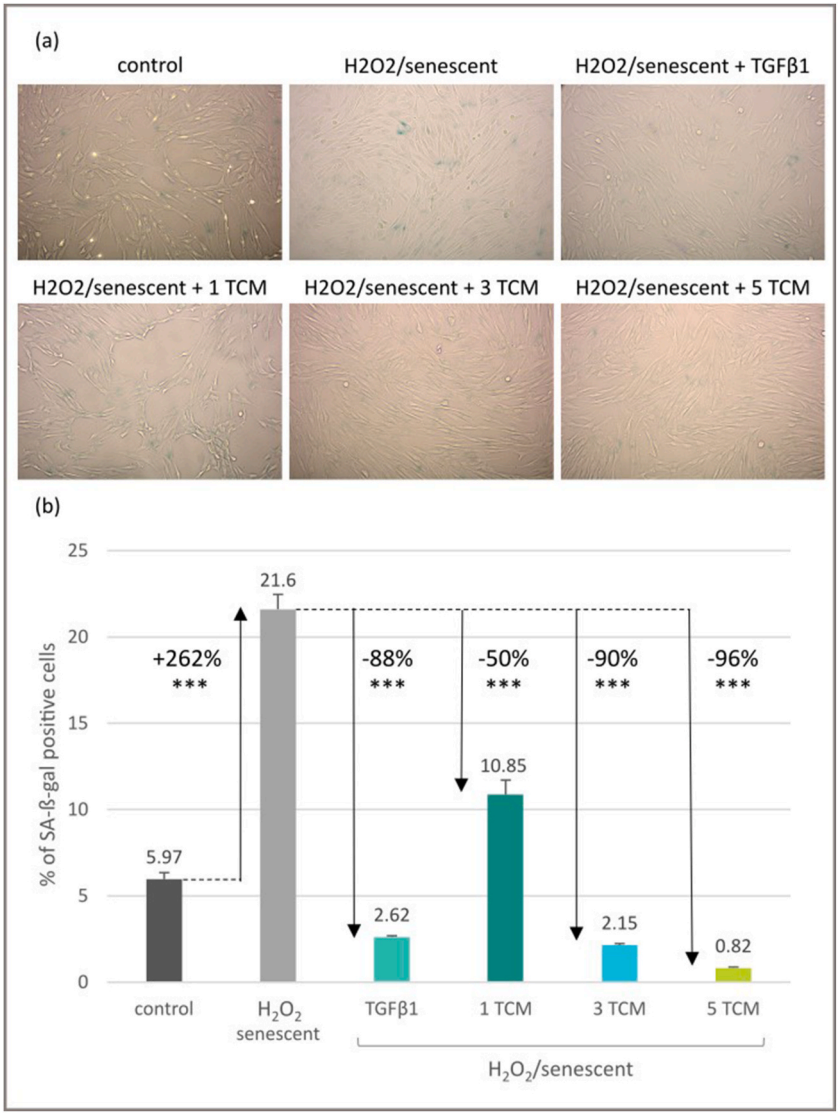

Fig. 4. (a) SA-β-gal positive cells (x100) in the H_2O_2 -stressed fibroblasts after 24h of incubation with TCM combinations or TGFβ1. (b) The percentage of SA-β-gal positive cells in each condition. Statistical analysis was carried out with the Student's *t*-test compared to control condition for H_2O_2/senescent condition and to the H_2O_2/senescent condition for the other conditions. (n = 3, ***: highly significant).

an equivalent efficacy than the 3 TCM (−39%)

4. Discussion & conclusions

TCM is a Chinese practice that has existed for centuries and is a reference for using Chinese herbal medicines to restore the yin yang balance for a healthy and harmonious human body. In TCM, herbal formulas are used considering the specific properties of each herb to improve the state of the human body. In this study, different plants with known potential related to skin improvement, were used in different combinations. The *in silico* study allowed us to predict a strong anti-aging and anti-senescence potential, for the three different plant extracts combinations, revealed by the following terms: "cellular senescence", "longevity regulating pathway" and "aging". The antioxidant response was also predicted with the term "oxidative stress response" for all TCM combinations. A better predictive impact of the 5 TCM combination was shown. These predicted activities were confirmed *in vitro* during the assessment of the effect of 1TCM, 3 TCM and 5 TCM combinations for their capabilities to reverse H_2O_2 induced senescence in fibroblasts. Moreover, the level of SA-β-gal positive cells decreased in H_2O_2/senescent cells when treated with 1 TCM, 3 TCM and 5 TCM combinations, with a better significance for the 5 TCM. Additional activities were also predicted with *in silico* analysis for the 3 TCM and 5 TCM combinations: the protection of DNA, with a better significance for the 5 TCM. *In vitro* study of γH2AX protein in H_2O_2/senescent cells showed an improvement of the induced DNA damage, through a decrease of γH2AX protein, mainly with the 5 TCM combination. These results confirmed the

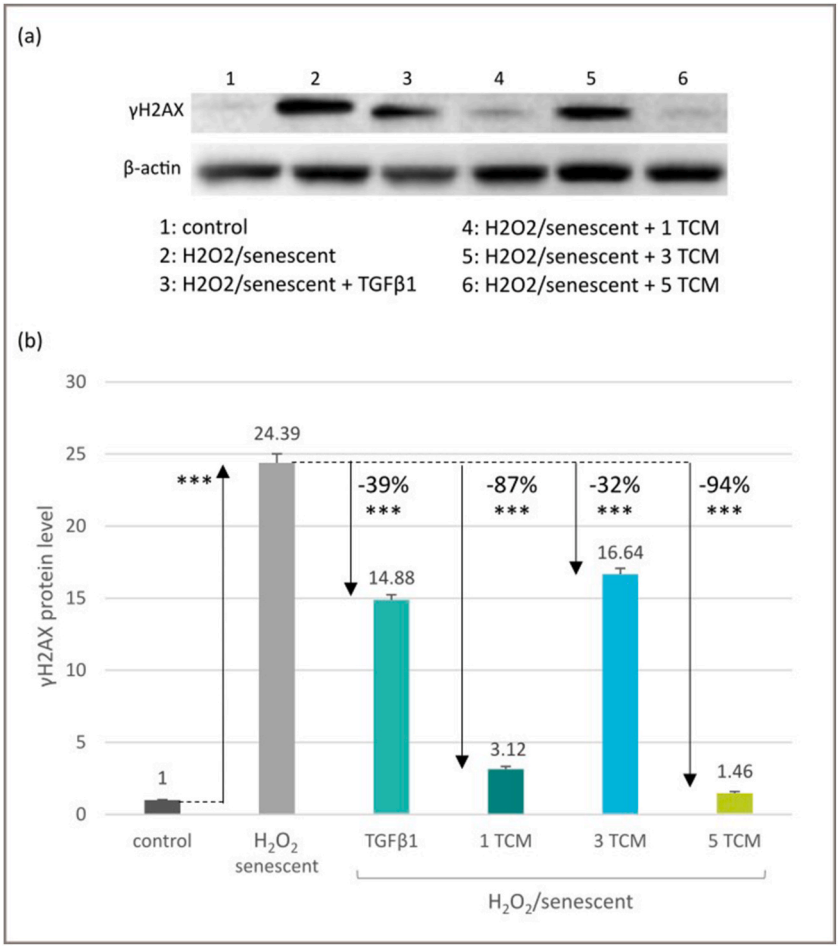

Fig. 5. (a) Evaluation of γH2AX expression in H_2O_2-senescent cells treated with TCM combinations using Western blot analysis. (b) γH2AX expression level in H_2O_2-senescent cells treated with TCM combinations. Statistical analysis was carried out with the Student's *t*-test compared to control condition for H_2O_2/senescent condition and to the H_2O_2/senescent condition for the other conditions. (n = 3,***: highly significant).

predicted performance of the 5 and 3 TCM combinations in the protection against DNA damage. Surprisingly, *Panax ginseng* alone showed good efficacy too while no evidence was predicted in the *in silico* study. In this case, the limitation of *in silico* predictions was highlighted. The *in silico* was performed based on some compounds of interest present in the plant extract but other non-identified compounds could explain its biological activity. Predictive anti-aging effect of the 3 and 5 TCM combinations, regarding anti-senescence and genomic stability, was thus accurate. Moreover, bioinformatic prediction showed a potential impact of each combination on dermal regeneration and extracellular matrix homeostasis with a better significance for the 5 TCM. This prediction was confirmed with the increase of collagen I and III in fibroblasts with also a better effect of the 5 TCM. The wide range of predictions revealed other potential axes of study in the domain of aging and skin biology: response to xenobiotic and pollutants, autophagy regulation, mitochondrial homeostasis, proteolysis, ubiquitination, proteasome functions, histone demethylase activity, epidermal homeostasis, cell adhesion and junction, skin barrier and water transport, hyaluronan homeostasis, glycosaminoglycans, adipocyte homeostasis, lipid metabolism, vitamins homeostasis and metabolism, skin tone modulation, melatonin metabolism, HSP90 and cannabinoid pathways. The global accuracy of *in silico* predictions allowed us to hypothesize that the 5 TCM combination is the best formula to fight against skin aging and improve skin homeostasis and, finally, the *in vitro* results confirmed this potential.

Target prediction is a key tool for understanding the mechanism of action of phytochemical compounds present in TCM plants. In this study, the robustness of the prediction was demonstrated by confirming the biological effects described in the literature for the evaluated TCM plants and by validating the effects identified in the presented *in vitro* analysis. In addition, target prediction is a predictive approach with low computational cost, robustness, and efficiency compared to experimental screening and it continues to progress.

However, *in silico* analysis has limitations that depend on the availability of chemical and biological data in the bioinformatics tool. In this study, we limited the *in vitro* evaluation to a few biological processes identified by the *in silico* prediction. Further experiments are required to validate the other predicted effects.

To conclude, the *in silico* analysis is a relevant tool to help define the better plant extracts combination to enhance the anti-aging

activities and finally providing a large source of data regarding biological activities potentially targeted. Finally, *in vitro* studies of the five plant extracts showed a real impact in the fighting of skin aging and the improvement of skin homeostasis, with the best results obtained with the 5 TCM combination.

Data availability

Data included in article/supplementary material/referenced in article.

CRediT authorship contribution statement

Chunbo Feng: Validation, Resources, Project administration, Methodology, Investigation, Conceptualization. **Catherine Serre:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation. **Weimiao Chen:** Writing – original draft, Investigation. **Isabelle Imbert:** Validation, Supervision. **Le Zhu:** Investigation, Conceptualization. **Feng Ling:** Methodology, Investigation.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, authors used [SwissTargetPrediction, DAVID] in order to predict biological targets of phytochemical compounds and to predict biological activities of several plant extracts. After using this tool, authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e26131.

ABBREVIATIONS

TCM	Traditional Chinese Medicine
DNA	Deoxyribonucleic acid
HPLC	High Performance Liquid Chromatography
MS	Mass spectrometer
NMR	Nuclear Magnetic Resonance
UV	Ultra Violet
IR	Infrared
1 TCM	*Panax ginseng* root extract
3 TCM	*Panax ginseng* root extract, *Polygonatum cyrtonema* rhizome/root extract and *Epiphyllum oxypetalum* flower extract combination
5 TCM	*Panax ginseng root extract, Polygonatum cyrtonema* rhizome/root extract, *Epiphyllum oxypetalum* flower extract, *Osmanthus fragrans flower* extract and *Nelumbo nucifera* extract combination
SMILES	simplified molecular-input line-entry system
DAVID	Database for Annotation, Visualization, and Integrated Discovery
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
NHDF	normal human dermal fibroblast
DMEM	Dulbecco's Modified Eagle Medium
PBS	Phosphate-buffered saline
TFGβ1	Transforming Growth Factor Beta 1
PCR	Polymerase chain reaction
COL1A1	Collagen Type III Alpha 1 Chain
COL3A1	Collagen Type I Alpha 1 Chain

SA-β-gal Senescence-associated beta-galactosidase
SDS-PAGE sodium dodecyl sulfate–polyacrylamide gel electrophoresis
HRP Horseradish peroxidase
AGE advanced glycation endproducts
RAGE receptor for advanced glycation endproducts
SASP Senescence-associated secretory phenotype
H3K9 histone H3 lysine 9
H3K36 histone H3 lysine 36
mRNA messenger ribonucleic acid
RNA ribonucleic acid
H_2O_2 Hydrogen Peroxide

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