Propylene Glycol Improves Stability of the Anti-Inflammatory Compounds in Scutellaria baicalensis Extract

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Abstract: Scutellaria baicalensis root extracts have been useful for external skin care and have been commercialized for years. Here, a comprehensive study was conducted to investigate the difference between 20% propylene glycol (PG) and water used as the storage solvent for a time course analysis of their remaining biological activities and ingredient compositions versus their freshly prepared conditions. Of the four major components in the 20% PG solvent, more than 80% of the components were retained after storage for two months, but in water only baicalin and wogonin were retained. The relative antibacterial activities, antioxidant properties and anti-inflammatory activities of the 20% PG solvent group were better than those of the water solvent group. Taken together, we demonstrated that these activities improved when PG, a common solvent used in many product formulas, was used as the storage solvent for the S. baicalensis extract.

Keywords: Scutellaria baicalensis; storage solvent; propylene glycol; anti-inflammation

1. Introduction

Scutellaria baicalensis Georgi (Huang-qin in Chinese) is one of the most widely used traditional Chinese medicines and is officially listed in the Chinese Pharmacopoeia. This plant is widely distributed, especially in China, Korea, Japan, Taiwan and India, and it is also found in Far East Russia, Siberia and Mongolia. In Taiwan, S. baicalensis has been introduced as a medicinal plant [1] and is now planted under the Taiwan Good Agriculture Practice (TGAP). S. baicalensis contains various bioactive flavonoid components, and the four major bioactive components are baicalin, its aglycone baicalein, wogonoside and its aglycone wogonin [2]. These flavonoids have a wide variety of physiological activities, whether they are taken internally or externally. Recently, S. baicalensis extracts have attracted interest worldwide for their external skin care and cosmeceutical benefits.

In a skin care application study, it was found that the S. baicalensis extract offered protection against ultraviolet A (UVA)-induced aging responses from skin fibroblasts, such as a reduction in telomere length, increased malonyldialdehyde (MDA) formation and expression of the enzyme matrix metalloproteinase-1 (MMP-1) [3]. It has also been observed that the S. baicalensis extract promotes keratinocyte differentiation and helps restore skin barrier function via activation of peroxisome proliferator-activated receptor (PPAR)-α [4]. Furthermore, S. baicalensis presents antioxidant activity [5], anti-inflammatory activity [6] and antibacterial activity [7], thus showing the potential use of the S. baicalensis extract as an antiallergic and anti-inflammatory agent for skin care. In the cosmeceutical industry, functional extracts are usually sold in a liquid form in order to facilitate distribution throughout the product. The chemical structures of the main bioactive components of...
S. baicalensis include baicalin, baicalein, wogonoside and wogonin (Figure 1), which are lipid-soluble substances. Thus, ethanol and other organic solvents must be used for extraction, but organic solvents are not desirable for skin care development [8]. Therefore, Ni et al. [8] and Choi et al. [9] used water as the extraction solvent to optimize the water extraction process of S. baicalensis.

![Chemical structures of baicalin, wogonoside, baicalein and wogonin.](image)

Figure 1. Chemical structures of baicalin, wogonoside, baicalein and wogonin.

Stability investigations are often sought by manufacturers to study degradation, which is usually caused by time, temperature, pH, moisture, light, storage solvent, etc., and their relative impacts on the quality of functional extracts [10]. Xing et al. [11] found that baicalin is stable in acidic and organic solutions but unstable in basic solutions and that adding vitamin C and HCl to baicalein samples can improve their stability in biological fluids. Qiu et al. [12] reported that a relatively lower temperature is conducive to baicalein stability. Feng et al. [13] published a detailed report on the stability of baicalin and baicalein in phosphate buffer and biological fluids in vitro and showed that acidic environments (pH 2–4.5), lower temperatures (<4 °C) and acidic antioxidants (e.g., vitamin C) were conducive to stabilizing them. Based on the above literature, we know that current research on stability is focused on the degradation or biotransformation in biological fluids, as well as pH and temperature changes. Moldovan et al. [14] demonstrated that storage solvent is one of the important factors affecting degradation because phenolic compound storage in water or ethanol can result in different degradation processes. However, to date, information is not available in the literature regarding the degradation of the bioactive components of S. baicalensis, including baicalin, baicalein, wogonoside and wogonin, in different storage solvents. Although some solutions have overcome the degradation problems of individual components of the S. baicalensis extract, workable storage options remain an unmet need for industrial use, not only concerning component stability but also to maintain the extract functions.

Propylene glycol (PG) is a synthetic liquid substance that absorbs water and is used by the chemical, food, and pharmaceutical industries. The Food and Drug Administration (FDA) has classified PG as an additive that is “generally recognized as safe” for use in food. It is used to absorb extra water and maintain moisture in certain medicines, cosmetics, and food products (https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=184.1666&SearchTerm=propylene%20glycol (accessed on 1 April 2020)).
The European Medicines Agency EMA/CHMP/704195/2013 (published 09/10/2017) [15] reported that PG is used as a humectant in topicals (15%), as a preservative in solutions (15–30%) or as a co-solvent in aerosols (10–25%). The usual concentration of PG in external products is between 10 and 30%, so we choose the median value of 20% PG as the storage solvent in the present study. To predict the quality changes of the bioactive components of *S. baicalensis* during storage, our study used 20% PG and water as the storage solvents to determine the changes in antibacterial activity, antioxidiant activity, anti-inflammatory activity, phototoxicity potential and comparison of baicalin, baicalein, wogonoside and wogonin contents. The conditions investigated here ensure high compatibility with storage and processing techniques generally applied in the skin care and pharmaceutical industries.

2. Materials and Methods

2.1. Plant Material, Reagents and Media

The plant material *S. baicalensis* used in this study was cultivated and produced by following Taiwan Good Agricultural Practice (TGAP) guidance. The dry roots of *S. baicalensis* were kindly provided by Dr. Tung-Wu Chang (Hualien District Agricultural Research and Extension Station Council of Agriculture, Executive Yuan, R.O.C.). Dulbecco’s phosphate-buffered saline (DPBS) and Hank’s balanced salt solution (HBSS, 21-023-CRV) were purchased from Corning (Manassas, VA, USA). PG was purchased from 63 PURE CHEMICALS (Taipei, Taiwan) with a purity >= 98%. Baicalin (cat. no CFN99111), wogonoside (cat. no CFN99710), baicalein (cat. no CFN98783) and wogonin (cat. no CFN97089) were purchased from ChemFaces (Wuhan, China) with purities >= 98%. All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Preparation of the *S. baicalensis* Extract (SBE) and Storage Conditions

Five hundred grams of the dry roots of *S. baicalensis* were ground to a powder with particle sizes of less than 2 mm. The dry root powder was extracted with 60 liters of RO water at 80 °C for 2 h. The extract was freeze-dried until the water content was less than 1% in the final *S. baicalensis* extract (SBE) powders. The yield was 36.33% based on the dry weight of the *S. baicalensis* roots. For the storage test, a stock solution of the abovementioned SBE was prepared at a concentration of 10 mg/mL in distilled water or 20% PG. Both stock solutions were sterilized by passage through a 0.22 µm polyethersulfone (PES) filter, aliquoted into six 1 mL Eppendorf tubes and then stored in the dark at room temperature until the day for assays. In this study, we prepared three independent samples of each condition and storage time in order to reduce the impact of changes from external environmental factors and pollution during sample unpacking.

2.3. Characterization of the Bioactive Compounds from *S. baicalensis* by High Performance Liquid Chromatography (HPLC)

Ten micrograms of each SBE stock solution were prepared for analysis by a Waters HPLC system, which consisted of a pump controller (Waters 600), an autosampler (Waters 717 plus) with cooler, a photodiode array detector (Waters 996) and a Prodigy ODS (2) column (5 µm, 150 × 3.2 mm, 150 Å, Phenomenex) with a guard column (5 µm, 20 × 4 mm, YMC-Pack Pro C18). The mobile phase was composed of buffer A (acetonitrile with 0.1% trifluoroacetic acid (TFA) (v/v)) and buffer B (distilled water with 0.1% TFA (v/v)) with a gradient elution program of 10~57% buffer A (0–25 min), 57~57% buffer A (25–30 min), 57~90% buffer A (30–37 min) and 90~10% buffer A (37–38 min). The flow rate was 1.0 mL/min, and the detection wavelength was 280 nm. For quantitative determinations, the standard curves of baicalin, wogonoside, baicalein and wogonin were established in the range of 0.5 to 25 µg against the relative peak areas, with linear regression coefficients of > 0.997. The standard solution was prepared in dimethyl sulfoxide at a concentration of 5 mg/mL for each compound.
2.4. Antibacterial Activity

*Propionibacterium acnes* (BCRC 10723) and *Staphylococcus aureus* (BCRC 14980) (Bioresource Collection and Research Center, Taiwan) were cultured in Reinforced Clostridial Medium under anaerobic conditions in a Gas-pak at 37 °C or in Tryptic soy broth under aerobic conditions at 37 °C, respectively. A total of 100 µL of SBE stock solution (10 mg/mL) was added to an inoculum containing 100 µL of bacteria in a 96-well plate. After 24 h of incubation, the optical density (OD) of the culture medium was measured using a spectrophotometer at a wavelength of 600 nm. The relative antibacterial activity (R%) was calculated as follows: \( R\% = \frac{OD_{growth \ medium} - OD_{sample}}{OD_{growth \ medium}} \times 100\% \).

2.5. Antioxidant Activity by the 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Method

The DPPH radical scavenging determination was performed according to the method described by Okawa et al. [16]. Equal volumes of the test sample and DPPH working reagent were added to a 96-well plate, gently mixed by pipetting and then left to stand for 30 min in the dark at room temperature. After incubation, the absorbance of the plate was measured at 517 nm with a plate reader (Sunrise, TECAN, Switzerland). A mixture of the tested sample and pure methanol and a mixture of DPPH working reagent and sample diluted solvent served as the blank and blind controls, respectively. The OD of the assay was measured using a spectrophotometer at a wavelength of 517 nm. The equation used to calculate DPPH scavenging was as follows: DPPH scavenging% = \[ 1 - \frac{OD_{SBE \ stock} - OD_{blank}}{OD_{blind}} \] \times 100%. The EC50 value was determined by using GraphPad Prism version 5 software and analyzed by nonlinear regression (curve fit).

2.6. Cell Culture

Human keratinocytes (ATCC CRL-2309) were grown and maintained in Defined Keratinocyte-serum-free medium (SFM) (Gibco 10744-019) with Defined Keratinocyte-SFM Growth Supplement, and the concentrations added followed the manufacturer’s instructions. The plates were incubated at 37 °C in a humidified atmosphere with 5% CO2.

2.7. Intracellular Reactive Oxygen Species (ROS) Assay

Assessment of the intracellular oxidative stress was conducted by using the cell-permeable fluorescent probe 2′, 7′-Dichlorodihydrofluorescein diacetate (DCFH-DA), as described by Sittisart et al. [17], with certain modifications. Cells were seeded at 1.2 × 10^4 per well in a 96-well plate and incubated for 24 h with SBE stock solution (final concentration 50 µg/mL). Then, 20 µM DCFH-DA prepared in HBSS was added, and the cells were incubated in the dark for an additional 30 min. DCFH-DA was removed by washing the cells with HBSS twice. Then, 250 µM tBuOOH was added, and the intensity of the fluorescence signal was immediately detected by a FLUOstar Omega microplate reader (BMG Labtech, Germany) with an excitation wavelength of 485 nm and an emission wavelength of 520 nm. A time-dependent mode was used to record alterations in the fluorescent intensity over 60 min at 37 °C. The cells without stimulation by tBuOOH served as the control.

2.8. IL-1β Secretion

Cells were seeded at 1.2 × 10^4 per well in a 96-well plate and treated with 250 µM tBuOOH with or without SBE stock solution (final concentration 50 µg/mL) for 24 h. Cells treated without tBuOOH and SBE stock were utilized as the control. The concentration of IL-1β was determined by using an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems Inc., Minneapolis, MN) according to the manufacturer’s instructions.
2.9. Phototoxicity Potential

The phototoxic potential assay for keratinocytes was performed according to the method described by Amaral et al. [18] with certain modifications. Cells were seeded at $1.2 \times 10^4$ per well of a 96-well plate and treated with the photosensitive agent chlorpromazine (CPZ) (final concentration 9 µg/mL) and SBE stock solution (final concentration 50 µg/mL) for 1 h. Then, one plate was exposed to UVA light (1 J/cm$^2$) by using VILBER LOURMAT, BIO-LINK® BLX-365 Crosslinker, and the other plate was kept in the dark as a nonirradiated control. Unwanted light exposure was minimized carefully during the whole procedure. The cell survival index was calculated for the cell proliferation of each treatment and normalized to the control, which was culture medium only. Cell proliferation was analyzed using the MTS/PMS assay (Promega G5421). Cells were reacted with MTS/PMS for 4 h, and the absorbance was measured at 490 nm with a reference wavelength at 680 nm in a microplate reader (FLUOstar Omega, BMG Labtech, Germany).

2.10. Statistical Analysis

Statistical analysis of the data was performed using the one-way ANOVA and post hoc Tukey’s multiple comparison tests with GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA).

3. Results and Discussion

3.1. Comparative Stabilities of Saicalin, Wogonoside, Baicalein and Wogonin after SBE Storage in 20% PG and Water

For the storage test, SBE was prepared as 10 mg/mL stock solutions in water and 20% PG and then stored in the dark at room temperature for two months. The phytochemical composition of SBE and four flavonoid standards (baicalin, wogonoside, baicalein and wogonin) at different storage times are shown in Figure 2. The representative constituents of the SBE were baicalin, wogonoside, baicalein and wogonin, and the two storage solvents did not affect the four flavonoids within 24 h (0 month) but showed some degradation after one month. The concentrations of these four flavonoids were calculated using their standard curves. The major constituent of the SBE was baicalin. In the SBE, the concentrations of baicalin, wogonoside, baicalein and wogonin were 270.05 ± 2.71, 57.50 ± 4.32, 19.24 ± 1.01 and 8.61 ± 0.89 mg/g, respectively.

The storage stabilities of baicalin, wogonoside, baicalein and wogonin from the SBE stored in 20% PG and water are shown in Table 1. The profiles indicated no significant changes in baicalin content in the 20% PG solvent over two months. However, the baicalin content was significantly degraded in water after two months, and the retained % was 92.41 ± 0.84. There was a difference in the degradation of wogonoside between the two different storage solutions. In 20% PG, wogonoside began to degrade significantly after two months of storage (20% PG, two months, retained%: 80.85 ± 3.11). However, after storage in water for one month, the degradation of wogonoside (water, one month, retained%: 83.84 ± 2.10) was similar to that of 20% PG storage for two months. Baicalein showed strong degradation in water, with a retained % of less than 55% after one month, but it appeared to be more stable in 20% PG, with a retained % of 84.28 ± 1.80 at month 2. In both solvents, wogonin degraded as the storage time increased, and the solvents did not show significant differences after two months of storage. Our data show that the water solvent decreased the four major flavonoids in the order baicalein > wogonoside > wogonin > baicalin, and the 20% PG solvent slightly decreased the four major flavonoids in the order of wogonoside > baicalein, wogonin > baicalin. Among these components, 20% PG retained more than 80% of the contents after storage for two months. Therefore, 20% PG could maintain the stability of the four major flavonoids more effectively than water. It has been suggested that the solubility of flavonoids (such as hesperidin in lemon) is significantly higher in PG than in water [19]. With two hydroxyl groups, the results here show that PG is readily hydrated by surrounding water molecules through both hydrogen bond donation and acceptance [20]. Therefore, adding PG can lower the water activity to


avoid chemical reactions and microorganism growth and enhance the apparent solubility of the active ingredients [21].

Figure 2. HPLC chromatograms of baicalin, wogonoside, baicalein and wogonin from the Scutellaria baicalensis extract (SBE) stored in 20% PG or water for different numbers of months.
Table 1. The ingredient retained % of SBE storage for different months in 20% PG and water.

| SBE (mg/g) | 20% PG (Retained%) | Water (Retained%) |
|------------|---------------------|------------------|
|            | Storage time        | 0 month | 1 month | 1.5 months | 2 months | 0 month | 1 month | 1.5 months | 2 months |
| Baicalin   | 270.05 ± 2.71       | 100.00   | 98.25 ± 2.16<sup>a</sup> | 98.89 ± 4.55<sup>a</sup> | 98.21 ± 1.98<sup>a</sup> | 99.20 ± 3.79<sup>a</sup> | 97.07 ± 2.95<sup>a</sup> | 92.41 ± 1.84<sup>b</sup> |
| Wogonoside | 57.50 ± 4.32        | 100.00<sup>a</sup> | 97.61 ± 5.93<sup>a</sup> | 96.15 ± 6.39<sup>a</sup> | 80.85 ± 3.11<sup>b</sup> | 83.84 ± 2.10<sup>b</sup> | 80.94 ± 3.66<sup>b</sup> | 72.33 ± 2.13<sup>c</sup> |
| Baicalein  | 19.24 ± 1.01        | 100.00<sup>a</sup> | 98.19 ± 2.14<sup>a</sup> | 92.23 ± 6.87<sup>b</sup> | 84.28 ± 1.80<sup>c</sup> | 52.05 ± 3.89<sup>d</sup> | 47.18 ± 2.81<sup>d</sup> | 44.43 ± 8.09<sup>d</sup> |
| Wogonin    | 8.61 ± 0.89         | 100.00<sup>a</sup> | 99.89 ± 7.67<sup>a</sup> | 98.10 ± 1.70<sup>a</sup> | 83.82 ± 2.21<sup>c</sup> | 98.93 ± 1.90<sup>a</sup> | 92.76 ± 5.93<sup>b</sup> | 87.52 ± 2.05<sup>c</sup> |

The data are presented as the mean ± SD (n = 3). Letters indicate significant differences within each experiment at p < 0.05.

3.2. Effects of SBE Storage in 20% PG and Water on the Antibacterial Activities against P. acnes and S. aureus

*P. acnes* and *S. aureus* live in close proximity to each other on human skin, and both bacterial species can be isolated from acne vulgaris-affected skin sites. *P. acnes*, a gram-positive anaerobic bacterium, may play a major role in the initiation of the inflammatory reaction by stimulating the secretion of cytokines [22]. *S. aureus*, a Gram-positive aerobic bacterium, shows increased numbers in acne lesions [23]. A disturbed microorganism balance can impact skin health and might initiate or support the events that lead to skin disorders [24]. For the application of skin care products, we first analyzed the antibacterial activities of the SBE.

Regardless of the 20% PG or water solvent group, the relative antibacterial activities decreased over time. The relative anti-*P. acnes* activity of the water solvent group stored at months 1.0, 1.5 and 2.0 was significantly lower than that of the 20% PG solvent group (Figure 3A). Similar results were also shown for *S. aureus*, in which the water solvent group stored at months 1.5 and 2.0 had significantly lower relative antibacterial activity than the 20% PG solvent group (Figure 3B). Lu et al. [25] and Qiu et al. [26] both reported that baicalein has strong anti-*S. aureus* activity, and four flavones have anti-*S. aureus* activity in the order of baicalein > baicalin > wogonoside > wogonin [26]. This is presumably because of the significant decay of wogonoside and baicalein in the water solvent group during storage (Table 1), which reduces the antibacterial activity.

3.3. Effects of SBE Storage in 20% PG and Water on the Antioxidant Activity

Antioxidants have been shown to prevent the oxidative stress of free radicals associated with the pathogenesis of a number of chronic diseases, including inflammation-associated disorders [27]. A study has shown that higher antioxidant activities, based on DPPH radical scavenging, have an impact on the anti-inflammatory effects [28]. Therefore, we analyzed the DPPH radical scavenging activity of the 20% PG and water solvent groups to understand their anti-inflammatory potential. Figure 4 shows that with increasing storage time, the DPPH radical scavenging activity decreased, and the EC50 values for Log dilution-fold of the 20% PG group were higher than those of the water groups. Woźniak et al. [29] reported that the order of DPPH radical scavenging activity was baicalein > baicalin > wogonoside > wogonin, and the total antioxidant properties of *S. baicalensis* come mainly from baicalein with some contribution from baicalin, wogonoside and wogonin. It has been suggested that the SBE was more stable in 20% PG solution and maintained better antioxidant activity, and the decrease in DPPH radical scavenging activity might be related to the degradation of baicalein (Table 1).
Figure 3. The antibacterial activities against *P. acnes* (A) and *S. aureus* (B) of the *Scutellaria baicalensis* extract (SBE) stored in 20% PG and water for different numbers of months. The data are presented as the mean ± SD (n = 3). *p < 0.05 compared with the 20% PG solvent.
Woźniak et al. [29] reported that the order of DPPH radical scavenging activity was significantly better than that of the water group (Figure 5). Chi et al. [35] reported that *S. baicalensis* lowers IL-1β in subchronic and acute skin inflammation in vivo. It has been suggested that *S. baicalensis* can be a useful therapeutic agent for skin inflammatory disorders, and preparing a suitable solvent for short-term storage, such as 20% PG, could effectively retain its anti-inflammatory abilities. Furthermore, Olennikov et al. [36] have identified the structures of 125 phenolic compounds from *S. baicalensis*. Therefore, we suggested that 20% PG solvent not only maintained the stability of baicalin, wogonoside and wogonin, but also caused other phenolic substances to maintain their stability and to retain antioxidant activity and anti-inflammatory activity.

| Storage time (Month) | 0    | 0.5  | 1    | 1.5  | 2     |
|----------------------|------|------|------|------|-------|
| PG                   | 2.58 ± 0.03^a | 2.57 ± 0.01^a | 2.39 ± 0.02^b | 2.32 ± 0.02^c | 2.29 ± 0.01^c |
| Water                | 2.53 ± 0.01^a | 2.48 ± 0.02^b | 2.26 ± 0.02^c | 2.22 ± 0.01^c | 2.11 ± 0.02^d |

Figure 4. The antiradical activity (A) and EC50 (B) of the *Scutellaria baicalensis* extract (SBE) stored in 20% PG and water for different numbers of months. The data are presented as the mean ± SD (n = 3). Letters indicate significant differences within each group at p < 0.05.

3.4. Effects of SBE Storage in 20% PG and Water on Anti-Inflammatory Activity in Human Keratinocytes

Keratinocytes express and release inflammatory mediators in response to skin inflammation and pro-inflammatory cytokines such as IL-1β during the progression phase of the inflammatory process [30]. Ponec et al. [31] reported that more than 5% PG would affect the growth of keratinocyte, and Hoh and Maier [32] also reported that around 1317 mM PG (around 10% PG) causes a 50% decrease in absorbance of viable keratinocyte. In our present study, CRL-2309 cells were exposed to 0.1% PG under cell culture, so we suggested that the concentration of PG would not induce inflammation of CRL-2309 cells. Moreover, flavonoids with high reactive oxygen species (ROS) scavenging activity have been shown to exhibit anti-inflammatory activity [33]. The baicalein in 20% PG had significantly less decay and better antioxidant capacity than that in water after one month of storage (Table 1 and Figure 4B). Next, we induced keratinocyte inflammation with tBuOOH, a representative radical source in a biochemical study [34], and analyzed the ability of samples to inhibit IL-1β and intracellular ROS production.

The production of generated intracellular ROS and IL-1β was significantly elevated by tBuOOH in CRL-2309 cells. It was observed that 20% PG and water (50 µg SBE/mL) at month 0 could strongly inhibit intracellular ROS and IL-1β production. When the storage time was extended to one month, the inflammatory inhibition from the 20% PG group was significantly better than that of the water group (Figure 5). Chi et al. [35] reported that *S. baicalensis* lowers IL-1β in subchronic and acute skin inflammation in vivo. It has been suggested that *S. baicalensis* can be a useful therapeutic agent for skin inflammatory disorders, and preparing a suitable solvent for short-term storage, such as 20% PG, could effectively retain its anti-inflammatory abilities. Furthermore, Olennikov et al. [36] have identified the structures of 125 phenolic compounds from *S. baicalensis*. Therefore, we suggested that 20% PG solvent not only maintained the stability of baicalin, wogonoside and wogonin, but also caused other phenolic substances to maintain their stability and to retain antioxidant activity and anti-inflammatory activity.
suggested that *S. baicalensis* can be a useful therapeutic agent for skin inflammatory disorders, and preparing a suitable solvent for short-term storage, such as 20% PG, could effectively retain its anti-inflammatory abilities. Furthermore, Olennikov et al. [36] have identified the structures of 125 phenolic compounds from *S. baicalensis*. Therefore, we suggested that 20% PG solvent not only maintained the stability of baicalin, wogonoside and wogonin, but also caused other phenolic substances to maintain their stability and retain an antioxidant activity and anti-inflammatory activity.

![Figure 5.](image)

**Figure 5.** Effects of *Scutellaria baicalensis* extract (SBE) storage in 20% PG and water for one month on intracellular ROS (A) and IL-1β (B) production by tBuOOH stimulation in human keratinocytes. The data are presented as the mean ± SD (n = 3). Letters indicate significant differences within each experiment at *p* < 0.05.

### 3.5. Effects of SBE Storage in 20% PG and Water on the Phototoxicity Potential with Human Keratinocytes

Phototoxicity is a growing concern in dermatology due to stratospheric ozone depletion resulting in an increasing penetration of UV radiation. CPZ is a phototoxicant of keratinocytes. Upon UV irradiation, CPZ produces a variety of free radicals, including the corresponding cation radical, the neutral promazinyl radical, a chlorine radical and a super-centered peroxo radical. The neutral promazinyl radical can react covalently with the cell membrane, proteins and other macromolecules to yield antigens that could be responsible for the photoallergic response to CPZ, resulting in phototoxicity [37]. In the current study, CPZ was used as a positive control and, as expected, exhibited phototoxic activity in CRL-2309 cells. The effect of the solvent used in storage may be of concern to the alternating phototoxic risk of the SBE. As the results show, the application of 50 µg SBE/mL, 20% PG and water did not induce phototoxicity at months 0, 1 and 2 (Figure 6).
It was suggested that regardless of whether the SBE was stored in PG or water, it would not become a photosensitizing agent.

**Figure 6.** Evaluation of the phototoxic potential under UVA exposure of *Scutellaria baicalensis* extract (SBE) stored in 20% PG and water for different numbers of months to human keratinocytes. The data are presented as the mean ± SD (n = 3). Letters indicate significant differences within each experiment at *p* < 0.05.

### 4. Conclusions

Here, we have demonstrated that PG was a good choice of solvent for the short-term storage of the SBE for at least two months, and only the concentration of 20% used in this study could retain more than 80% ingredient availability, including baicalin, wogonoside, baicalein and wogonin. This solvent could also maintain anti-inflammatory activity and did not increase the phototoxic risk. PG is a common, safe and inexpensive additive for many industries. Thus, our findings provide a convenient and efficient way to improve the issues of SBE activity loss in storage and may extend further industrial applications.

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