

Effectiveness of Ginseng Berry Marc Extract for Skin Improvement

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Abstract

Purpose: This study identifies the possibility of ginseng berry marc as a cosmetic material for skin improvement through a physiological activation experiment. **Methods:** The measurement of the total polyphenol and flavonoid content, evaluation of antioxidants through DPPH radical scavenging activity, and cell viability ratio of 70% ethanol extract of ginseng berry marc extracts were conducted. To access the anti-inflammatory effects, NO generative inhibitory measurements and cell regeneration by cell migration assessment were investigated. **Results:** The total polyphenol content was 335 ± 12.3 mg/g, and the total flavonoid content was 79 ± 3.2 mg/g. DPPH radical scavenging activity also showed a concentration-dependent increase as the concentration increased to $37.73 \pm 2.47\%$ at 0.25 mg/mL, $49.33 \pm 0.66\%$ at 0.5 mg/mL, $73.56 \pm 3.56\%$ at 0.75 mg/mL, and $80.51 \pm 0.51\%$ at 0.9 mg/mL. The cell viability evaluation (MTT assay) was $93.33 \pm 1.88\%$ at 0.25 mg/mL, $90.66 \pm 1.24\%$ at 0.5 mg/mL, $88.66 \pm 1.69\%$ at 0.75 mg/mL, and $83.1 \pm 0.94\%$ at 0.9 mg/mL. At concentrations of 0.25 and 0.5 mg/mL, the cell survival rate is greater than 90%, and the lowest survival rate is greater than 80%, indicating that there is no problem with the safety of cytotoxicity. The anti-inflammatory evaluation showed that NO decreased as the concentration increased with 31.76 ± 0.52 μ M at 0.25 mg/mL, 21.76 ± 0.52 μ M at 0.5 mg/mL, 10.86 ± 0.47 μ M at 0.75 mg/mL, and 9.76 ± 0.28 μ M at 0.9 mg/mL. According to the comparison of cell movement and recovery conditions by concentration, the higher the concentration 2.5 mg/mL, 5 mg/mL, and 10 mg/mL, the faster space is filled, which can help the skin recover and regenerate. **Conclusion:** Eco-friendly natural ingredients can be obtained through upcycling of ginseng berry marc, which can be used as an effective skin-improving cosmetics ingredient through more studies.

Keywords: Ginseng berry marc, Skin improvement, Antioxidant, Anti-inflammatory, Skin regeneration

Introduction

Recently, the development of eco-friendly and natural cosmetics materials has been steadily growing in the cosmetics industry to improve skin worldwide. With the development of biotechnology, the demand for functional cosmetics containing natural ingredients is increasing. Consumers seek natural ingredient cosmetics to maintain human body protection and homeostasis from harmful environmental factors. (Ham et al., 2018).

Their so-called good values and consumption are seen as pursuing eco-friendly materials, and cosmetics materials of natural ingredients are especially recognized for their value. As a result, the fruits of plants and their extracts are steadily used as natural materials for cosmetics produced in Korea, and research on them is also continuously conducted. According to the results of the previous study, six berries types, including chokeberry, blueberry, raspberry, blackberry, mulberry, and cherry, were evaluated that the antioxidant and anti-inflammatory effects

were excellent, and all efficacy was increased when they were mixed (Kim & Kim, 2020). Furthermore, the prior studies' results on antioxidants (Song *et al.*, 2014), anti-inflammatory (Lee *et al.*, 2014), and anti-aging (Jeon *et al.*, 2011) effects proved that ginseng (*Panax ginseng* C.A. Meyer) berry extract is a safe nontoxic material. This berry type can be used as a natural functional cosmetics material with excellent antioxidant activity and anti-inflammatory efficacy (Kim & Ko, 2020). Another study has reported that ginseng berry has anti-cancer, anti-allergic, and anti-photo-aging effects, and skin aging improvement (Yeom *et al.*, 2010).

The ginseng berry marc is the leftover scraps after the use of ginseng berry. However, unlike various studies on ginseng berry, little research has been conducted on ginseng berry marc. In a previous study on ginseng marcs, it was found that fat-soluble ingredients such as sulfuric acid and anti-cancer activity remained similar to ginseng and can be used as an effective ingredient by its physiological active composition (In *et al.*, 2014). Ginseng berry marc is a natural material that can be used in beauty skin products' development, environmental awareness, and sustainable practices in Korea.

Therefore, this study evaluates the physiological activity (cell viability ratio, antioxidant, anti-inflammatory, and cell regeneration effect) of ginseng berry marc to identify the possibility of its use as a cosmetic material for skin improvement.

Methods

1. Experimental material

Ginseng berry marc used in this experiment, grown in Gimje-si, Jeollabuk-do, and dried under hot-air (Agricultural dryer; HDG-230T, Hyundai Enertec Co., Ltd., Korea), was gained as a byproduct after extraction of ginseng berry oil. 10% of ginseng berry marc were immersed in 70% ethanol solution, and the effective ingredient was extracted at 25°C for 6 h. Afterward, it was operated 20 min of the centrifuge (CS150NX; Hitachi Ltd, Tokyo, Japan) at 4°C to 4000 rpm, separated the upper layer solution, and filtered (ADVANTEC Co, Ltd, Japan) three times. Then, it was concentrated using a pressure reduction concentrator (Evaporator; Tokyo Rikakikai co, Ltd., Japan), frozen dry, and made the extract powder of ginseng berry marc as experimental material.

2. Antioxidant activity

1) Total polyphenol Content Measurement

1 mL of 1 N Folin-Ciocalteu agent was applied to a solution prepared with a concentration of 1 mg/mL of ginseng berry marc, after 5 min reaction and applying 1 mL of a 10% Na₂CO₃ solution, 1 h retaining the mixture at room temperature, and finally measured absorbance at 700 nm using a spectrophotometer (UV-2450; Shimadzu Co., Japan). The total polyphenol content was calculated from the standard curve obtained using Gallic acid (GAE; Sigma-Aldrich, USA).

2) Total flavonoid content

2 mL of Di-ethylene glycol (Sigma-Aldrich) and 0.02 mL of 1 N NaOH (Sigma-Aldrich) were added to the solution prepared with a concentration of 200 µg/mL of ginseng berry marc. The absorbance was measured at 420 nm using a spectrophotometer (UV-2450; Shimadzu Co.) after retaining for 37°C and 1 h. The total flavonoid content was measured using quercetin (Sigma-Aldrich) as a standard substance by creating a correction curve.

3) DPPH radical scavenging activity

For antioxidant measurements, 2,2-Diphenyl-1-picrylhydrazyl (DPPH; Sigma-Aldrich) radical scavenging activity was performed using the Blois method (Blois, 1958). Added 1 mL of 0.2 mM DPPH solution (Sigma-Aldrich) to 2 mL of the sample and measured absorbance at 517 nm using a spectrophotometer (UV-2450; Shimadzu Co.) after 30 min in the darkroom. The radical scavenging activity was calculated using the following formula.

$$\text{DPPH radical scavenging activity (\%)} = [1 - (B/A)] \times 100$$

A: Absorbance of controls with only DPPH solution

B: Absorbance of DPPH solution with the sample solution

3. Cell viability evaluation (MTT assay)

A cytotoxicity test, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, was performed on ginseng berry marc extracts. For the cytotoxicity test, the concentration of the RAW 264.7 cell specimen was arranged and treated at 0.25 mg/mL, 0.5 mg/mL, 0.75 mg/mL, and 0.9 mg/mL. Then, 96-well plates were injected with 1 × 10⁴ cells/mL each and cultured for 24 h. After incubation, each well was treated with the diluted extract and incubated for 24 h. After removing 100 µL of cultured fluid, 10 µL of MTT solution was added to each well and reacted for 4 h at 37°C and 5%

CO₂ incubators. The MTT solution was removed, and 200 µL dimethyl sulfoxide (DMSO; Sigma–Aldrich) was used into each to dissolve the formazan crystals to measure 570 nm absorbance with ELISA plate reader (Bio Tec Ex800, USA). The cell viability ratio was calculated as a percentage (%).

4. Anti-inflammatory evaluation (NO assay)

For conducting the anti-inflammatory assessment, RAW 264,7 cells (mouse monocyte macrophages) from the Korea Cell State Bank Biological Resource Center were used to measure the production inhibitory activity of nitric oxide (NO). Raw 264.7 cells were injected into each and incubated at a concentration of 3×10⁴ cells/well for 24 h at incubator (37°C, 5% CO₂). Extracts were treated in each well with various concentrations of 0.25 mg/mL, 0.5 mg/mL, 0.75 mg/mL, and 0.9 mg/mL. The equivalent amounts of LPS 1 µL/mL were treated and incubated at incubator (37°C, 5% CO₂) for 24h. After adding 100 µL of cultured fluid and equivalent greasing reagent, reacting for 10 min, the absorbance was measured at 540 nm with the ELISA plate reader (BioTec Ex800, USA). Standard was quantified using NaNO₂.

5. Cell regeneration evaluation

The in vitro scratch assay (Liang *et al.*, 2007) method was used to test cell regeneration of ginseng berry marc extracts using HaCaT cells. These cells were injected into each at a concentration of 5×10⁴ cells/mL at 48 well plates. They were incubated for 24 h in a cell culture machine (37°C, 5% CO₂) and were scratched using a p200 pipette tip in the cell fault. 100 µL of the elution of each concentration (2,5 mg/mL, 5 mg/mL, 10 mg/mL) of ginseng berry marc was treated and incubated for 24 h. The status of cell migration and recovery was compared through an Inverted microscope (CKX53; Olympus–Scope, Japan) by different extract concentrations.

6. Statistics processing

The experimental results were analyzed with SPSS statistics 24 (IBM, USA), which obtained the mean and standard deviation. Student's *t*-test was conducted and Post-test for

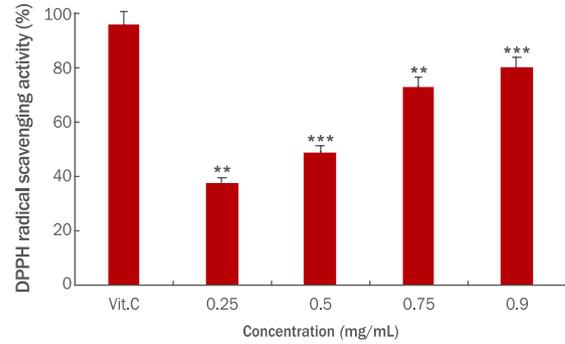


Figure 1. DPPH radical scavenging activity of ginseng berry marc ethanol extract.

DPPH radical scavenging analysis was performed to investigate. The antioxidant effects of ginseng berry marc at varying concentration levels of 0.25 mg/mL, 0.5 mg/mL, 0.75 mg/mL, 0.9 mg/mL. Each value presents the mean±standard deviation (n=3). Statistically significant differences are marked with an asterisk (**p*<0.05, ***p*<0.01, ****p*<0.001).

the mean difference was determined to be significant at 5% of *p*-value (*p*<0.05).

Results and Discussion

1. Total polyphenol and flavonoid content

This study examined the antioxidant efficacy by measuring the total polyphenol and flavonoid content present in ethanol extract of ginseng berry marc. The result shows that the total polyphenol content of ginseng berry marc extract was 335±12.3 mg/g, and the total flavonoid content was 79±3.2 mg/g (Table 1). These results are higher than that of polyphenols (44.1–178.3 mg GAE/g dw) and flavonoids (4.1–40.3 mg QE/g dw) indicated in previous studies of fruit skin antioxidant activity (Lee *et al.*, 2012).

2. DPPH radical scavenging activity

The DPPH measurement method used to check the antioxidant of ginseng berry marc extract is a method to determine the DPPH radical scavenging activity. The control

Table 1. Total polyphenol and flavonoid contents of ethanol extracts of ginseng berry marc

	Total polyphenol contents (GAE mg/g)	Total flavonoid contents (TA/100g)
Ginseng berry marc	335±12.3	79±3.2

Mean±SD, (n=3).

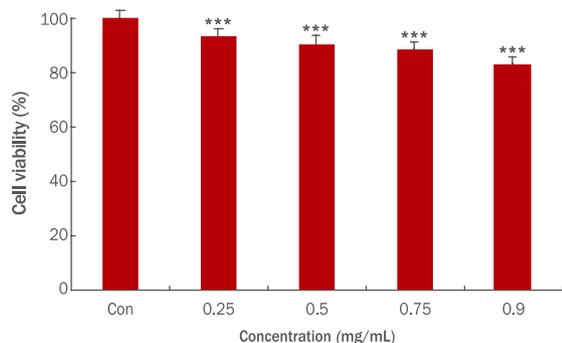


Figure 2. Effect of ethanol extracts on ginseng berry marc on cell viability of RAW 264.7 cells.

RAW 264.7 cells were treated with ethanol extracts on ginseng berry marc at varying concentration levels 0.25 mg/mL, 0.5 mg/mL, 0.75 mg/mL, 0.9 mg/mL. The values are expressed as the mean±standard deviation of three individual experiments. Statistically significant differences are marked with an asterisk (***) $p < 0.001$.

group handled with ascorbic acid 1 mg/mL, seen in Figure 1, had a DPPH radical scavenging activity of 96.2%. The concentration of ethanol extract from ginseng berry marc shows 37.73%±2.47% at 0.25 mg/mL, 49.33%±0.66% at 0.5 mg/mL, 73.56%±3.56% at 0.75 mg/mL, and 80.51%±0.51% at 0.9 mg/mL. These results show a concentration-dependent increase as the concentration increased and high antioxidant activity. As reported in previous studies, the hexane extract of ginseng marc obtained from ginseng is a byproduct with approximately the same level of antioxidant and anti-cancer activity as its efficacy in ginseng (In *et al.*, 2014). The high DPPH radical scavenging activity indicates that ginseng berry marc extract has an antioxidant effect in skin improvement (Figure 1).

3. Cell viability evaluation (MTT assay)

The results of the test to determine the cell viability ratio of ginseng berry marc extract are presented in Figure 2. When evaluated in concentration ranges of 0.25 mg/mL, 0.5 mg/mL, 0.75 mg/mL, 0.9 mg/mL, the cell viability ratio was 93.33%±1.88%, 90.66%±1.24%, 88.66%±1.69%, and 83.10%±0.94%, respectively. The cell survival tended to decrease slightly as ginseng berry mark extracts went from concentration of 0.25 mg/mL to 0.9 mg/mL. However, at concentrations of 0.25 and 0.5 mg/mL, the cell survival rate is greater than 90%, and the lowest survival rate is greater than 80%, indicating that there is no problem with the safety of cytotoxicity.

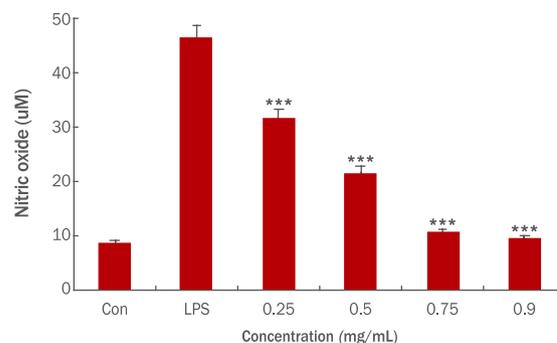


Figure 3. Effects of ethanol extracts on ginseng berry marc on LPS-induced nitric oxide production RAW 264.7 cells.

RAW 264.7 cells were treated at the indicated concentrations of ginseng berry marc extracts and treated with LPS (1 µg/mL). After 24 h of incubation, the amounts of nitric oxide were measured by Griess reaction assay. Results are represented as mean±standard deviation. Statistically significant differences are marked with an asterisk (***) $p < 0.001$.

4. Anti-inflammatory evaluation (NO assay)

An experiment nitrite oxide (NO) was conducted to verify the anti-inflammatory efficacy of ginseng berry marc extracts, and the measurement of the control group was 8.83±0.46 µM. The amount of NO produced by LPS at a concentration of 46.67±1.54 µM shows that the concentration of ginseng berry marc extract decreased, respectively, 31.76±0.52 µM at 0.25 mg/mL, 21.76±0.52 µM at 0.5 mg/mL, 10.86±0.47 µM at 0.75 mg/mL, and 9.76±0.28 µM at 0.9 mg/mL. As the concentration of ginseng berry marc extract increased, NO production decreased (Figure 3). So, ginseng berry extract was found to have an anti-inflammatory effect and relieve inflammation. Prior research revealed that the ingredients of polyphenolic substances at the ground level of ginseng fruits have a major effect on anti-inflammatory efficacy rather than saponin, also contained in ginseng berry (Lee *et al.*, 2014). Therefore, ginseng berry marc extract has an anti-inflammatory effect, being an effective cosmetic material effective in improving inflammatory skin.

5. Cell regeneration evaluation

To determine how ginseng berry marc extract affects skin improvement, HaCaT cells were injected into each and cultured, treated the extract by concentration (2.5 mg/mL, 5 mg/mL, and 10 mg/mL), and checked whether cell migration was active in the space created. Through an inverted microscope (CKX53; Olympus-Scope), cell movement and recovery conditions were

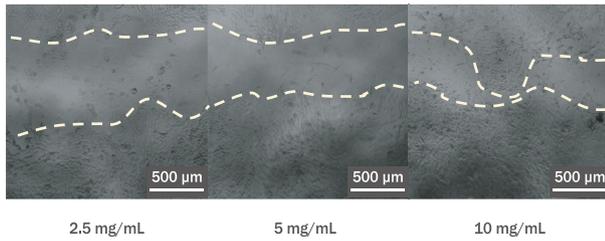


Figure 4. Effect of ginseng berry marc ethanol extracts on cell migration.

HaCaT cells were concentrated on 48 well plates at a concentration of 5×10^4 cells/mL, stabilized at a cell culture medium (37°C , $5\% \text{CO}_2$), scratched the cell layer for 24 h to create space.

compared with different extract concentrations. The results (Figure 4) indicated that the higher the concentration, the faster space is filled, and the narrower the area can be. Prior studies of ginseng berry extracts have shown efficacy in promoting the production of hyaluronic acid, which is highly involved in water retention, intercellular spacing, cell division, and differentiation movement in the skin (Yeom *et al.*, 2010). In addition, this result indicates that it is a material that can help the skin recover and regenerate.

Conclusion

The present study examined the total polyphenols and flavonoids content to determine the physiological activity (antioxidant, anti-inflammatory, and cell regeneration) of ginseng berry marc extracts. In addition, were conducted several experiments, such as antioxidant activity (DPPH), cell viability evaluation (MTT assay), anti-inflammatory evaluation (NO assay), and cell regeneration evaluation to determine whether ginseng berry marc extracts improve the skin.

As a result, the total polyphenol content of ginseng berry marc was 335 ± 12.3 mg/g, and the total flavonoid content was 79 ± 3.2 mg/g. The antioxidant effect (DPPH radical scavenging activity) also showed a concentration-dependent increase as the concentration increased to $37.73\% \pm 2.47\%$ at 0.25 mg/mL, $49.33\% \pm 0.66\%$ at 0.5 mg/mL, $73.56\% \pm 3.56\%$ at 0.75

mg/mL, and $80.51\% \pm 0.51\%$ at 0.9 mg/mL. The cell viability evaluation (MTT assay) was $93.33\% \pm 1.88\%$ at 0.25 mg/mL, $90.66\% \pm 1.24\%$ at 0.5 mg/mL, $88.66\% \pm 1.69\%$ at 0.75 mg/mL and $83.1\% \pm 0.94\%$ at 0.9 mg/mL. At concentrations of 0.25 and 0.5 mg/mL, the cell survival rate is greater than 90%, and the lowest survival rate is greater than 80%, indicating that there is no problem with the safety of cytotoxicity. The anti-inflammatory evaluation (NO assay) showed that NO decreased as the concentration increased with 31.76 ± 0.52 μM at 0.25 mg/mL, 21.76 ± 0.52 μM at 0.5 mg/mL, 10.86 ± 0.47 μM at 0.75 mg/mL, and 9.76 ± 0.28 μM at 0.9 mg/mL. According to the extract concentration comparison of cell movement and recovery conditions, the higher the concentration, the faster space is filled, which can help the skin recover and regenerate.

The results of the physiological activation experiments show that ginseng berry marc has effectiveness in antioxidant, anti-inflammatory, and cell regeneration. In the near future, through more studies, eco-friendly natural ingredients can be obtained through upcycling, where these discarded natural byproducts can be used as an effective cosmetic ingredient for skin-improving.

Author's contribution

HSK designed and analyzed all experimental surveys, HSK wrote the manuscripts.

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국문초록

피부개선을 위한 인삼열매박 추출물의 효능

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목적: 본 연구에서는 인삼열매박의 피부개선을 위한 화장품 소재로의 활용 가능성을 규명하고자 생리활성을 실험을 진행하였다. **방법:** 인삼열매박 70% 에탄올 추출물의 총 폴리페놀, 플라보노이드 함량과 DPPH 소거활성 평가를 통한 항산화, 세포 생존율(MTT assay) 평가를 하였다. 또한 NO 생성 저해 측정을 통해 항염 효과를 알아보고 세포이동률 평가를 통해 세포 재생에 관하여 알아보았다. **결과:** 총 폴리페놀 함량은 335 ± 12.3 mg/g로 나타났으며, 총 플라보노이드 함량은 79 ± 3.2 mg/g로 나타났다. DPPH free radical 소거능은 인삼열매박 추출물 농도 0.25 mg/mL에서 $37.73\% \pm 2.47\%$, 0.5 mg/mL에서 $49.33\% \pm 0.66\%$, 0.75 mg/mL에서 $73.56\% \pm 3.56\%$, 0.9 mg/mL에서 $80.51\% \pm 0.51\%$ 로 농도가 증가함에 따라서 소거능도 농도 의존적인 증가를 보였다. 세포 생존율 평가(MTT assay)는 농도 0.25 mg/mL에서 $93.33\% \pm 1.88\%$, 0.5 mg/mL에서 $90.66\% \pm 1.24\%$, 0.75 mg/mL에서 $88.66\% \pm 1.69\%$, 0.9 mg/mL에서 $83.1\% \pm 0.94\%$ 로 나타났다. 0.25와 0.5 mg/mL의 농도에서 세포 생존율이 90% 이상이며 가장 낮은 생존율도 80% 이상으로 세포독성에 대한 안전성에 전혀 문제가 없는 것으로 확인되었다. 항염증 평가에서 농도가 0.25 mg/mL에서 31.76 ± 0.52 μ M로 감소했으며, 0.5 mg/mL일 때 21.76 ± 0.52 μ M, 0.75 mg/mL일 때 10.86 ± 0.47 μ M, 0.9 mg/mL일 때 9.76 ± 0.28 μ M 감소시킨 것으로 나타났다. 세포이동과 회복의 상태를 농도별로 비교한 결과 농도가 2.5 mg/mL, 5 mg/mL, 10 mg/mL로 높아질수록 빈 공간을 채우는 속도가 활발하며 피부의 회복과 재생에 도움을 줄 수 있는 소재임을 확인하였다. **결론:** 인삼열매박은 생리활성 실험에서 항산화, 항염, 세포 재생의 활성을 갖는 것으로 나타났으며 버려지는 천연 부산물의 재활용을 통해 친환경적인 천연 성분으로 효과적인 피부개선 화장품 원료로 활용 가능할 것으로 기대된다.

핵심어: 인삼열매박, 피부개선, 항산화, 항염, 피부 재생

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中文摘要

人参浆果渣萃取物改善皮肤的功能

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目的: 这项研究通过测定人参生理活性来确定人参浆果渣作为美容材料改善皮肤的可性性。**方法:** 测定人参浆果渣提取物的总多酚和类黄酮的含量, 通过DPPH自由基清除活性评估抗氧化剂, 以及细胞生存率。为了获得抗炎作用, 测定了NO生成抑制并通过细胞迁移来评估细胞再生。**结果:** 测定结果如下: 总多酚含量为 335 ± 12.3 mg/g, 总黄酮含量为 79 ± 3.2 mg/g。DPPH自由基清除活性也显示出浓度依赖性的增加, 浓度在0.25 mg/mL时, 增加到 $37.73\% \pm 2.47\%$; 浓度为0.5 mg/mL时, 增加到 $49.33\% \pm 0.66\%$; 浓度为0.75 mg/mL时, 增加到 $73.56\% \pm 3.56\%$; 浓度为0.9 mg/mL时, 增加到 $80.51\% \pm 0.51\%$ 。细胞生存率 (MTT分析) 测定结果显示: 在浓度为0.25 mg/mL时, 为 $93.33\% \pm 1.88\%$, 在0.5 mg/mL时, 为 $90.66\% \pm 1.24\%$; 在0.75 mg/mL时, 为 $88.66\% \pm 1.69\%$; 在0.9 mg/mL时, 为 $83.1\% \pm 0.94\%$ 。在0.25和0.5 mg/mL的浓度下, 细胞存活率大于90%, 最低存活率也大于80%, 表明细胞毒性的安全性没有问题。抗炎评估显示, 随着浓度的增加, NO降低, 浓度为0.25 mg/mL时, 减少到 31.76 ± 0.52 uM, 0.5 mg/mL时, 减少到 21.76 ± 0.52 μ M, 0.75 mg/mL时, 减少到 10.86 ± 0.47 μ M, 0.9 mg/mL时, 减少到 9.75 ± 0.28 μ M。根据浓度对细胞运动和恢复条件的比较, 随着浓度增加到2.5, 5和10 mg/mL时, 填充空白空间的速度变得更加活跃, 并且可以确认这是一种有助于皮肤恢复和再生。**结论:** 人参浆果渣在生理活性实验中被发现具有抗氧化, 消炎和细胞再生的活性, 并建议通过回收废弃的天然植物将其用作有效的皮肤改善化妆品原料。

关键词: 人参浆果渣, 皮肤改善, 抗氧化, 抗炎, 皮肤再生