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New Functional Properties of Passion Fruit Extract on Skin

Mi Suk Jeong[1,3†], Soon-Rae Kim[2†], Chang Woo Han[1†], Hyeon Jin Kim[1,3†] and Se Bok Jang[1*]

[1]*Department of Molecular Biology, College of Natural Sciences, Pusan National University, 2, Busandaehak-ro 63 beon-gil, Geumjeong-gu, Busan 46241, Korea*
[2]*Department of Food Science & Nutrition, College of Fisheries Science, Pukyong National University, 45 Yongso-ro, Nam-gu, Busan 48513, Korea*
[3]*GJMEDI INC., Hyowon University-Industry Cooperation Bldg., 2, Busandaehak-ro 63 beon-gil, Geumjeong-gu, Busan 46241, Korea*

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In this research, the anti-inflammatory, antioxidant, antiaging, and skin whitening properties of pulp and seed extracts of passion fruit were studied. The result of the primary skin irritation test using a skin-attached patch determined the skin irritation index to be 0.00 for the passion fruit extract. In addition, RAW 264.7 macrophages produce NO by stimulation of lipopolysaccharides, and the application of extracts to this resulted in significantly lower NOs, confirming the excellent anti-inflammatory properties of passion fruit extracts. The 2,2-diphenyl-1-picrylhydrazyl test further confirmed that the passion fruit extract exhibits a good 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate radical scavenging ability of 5.11% and strong antioxidant properties. The presence of collagen type I in the skin is a measure of aging and various skin diseases. The results obtained from the analysis of the activity of human procollagen I alpha 1 confirmed that the passion fruit extract reduces the synthesis of procollagen. In addition, the skin whitening property of the passion fruit extract was confirmed by the melanin inhibition test, and a sample was obtained that contained more than 2% of arbutin, a whitening agent approved by the Ministry of Food and Drug Safety, which is generally present in the form of a white powder and is used as a functional ingredient. This confirms that the whitening efficacy of the passion fruit extract obtained from nature contributes to the development of functional raw materials for cosmetics and food.

Key words : Anti-aging, anti-inflammatory, antioxidant, passion fruit, whitening

Introduction

Living standards and average life expectancy have been extended along with the economic growth, resulting in greater efforts by the modern generation to manage and maintain their health and beauty. However, due to environmental pollution and destruction of the ozone layer, the skin is excessively exposed to ultraviolet rays, causing numerous skin afflictions including skin aging, pigmentation, erythema, and wrinkles, as well as impacting the mental balance. Recent years have seen an increased interest in natural substances, with natural foods being preferred to live a healthy life, and using products containing natural substances for maintaining beauty [5, 7, 8, 14, 15]. Accordingly, reflecting the increasing diverse needs of the consumer and their preference for cosmetics containing natural materials, research and development on functional natural materials are actively being undertaken [1, 3].

Passion fruit is a perennial evergreen plant native to Brazil and grown in subtropical and tropical regions. More than 500 species of Passifloraceae are distributed worldwide, and many are edible and used for ornamental purposes. The main varieties include "Rubista," "Red Purple," "New Black," "Peach," and "Black Beauty." The edible part resembles a table tennis ball with fruit, weighs 40-70 g, and has a good flavor and aroma, and is often used to make drinks, processed products, and perfumes [7, 20].

Passion fruit contains alkaloids, saponins, polyphenols, flavicarpa, lucenin-2, vicenin-2 (vicenin-2), isoorientin, isovitexin, luteolin-6-C-chinovoside, and luteolin-6-C-fucoside. In addition, passion fruit is a plant whose stability has been proven, with no adverse side effects being reported to date [16].

Previous studies have reported antithrombotic compositions containing passion fruit (*Passiflora edulis*) percutaneous extracts as active ingredients. These studies have determined

[†]**Authors contributed equally.**
***Corresponding author**
Tel : +82-51-510-2523, Fax : +82-51-581-2545
E-mail : sbjang@pusan.ac.kr
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the pharmaceutical compositions and health functional foods for the prevention or treatment/improvement of thrombosis through blood clotting inhibition, containing a passion fruit pulp extract prepared from the skin of water-cleaned passion fruits and fractionated into ethyl acetate. The ethyl acetate fraction of passion fruit skin extract as an active ingredient in thrombosis prevention or treatment and health functional foods exerts strong antithrombotic activity due to inhibition of the thrombotic enzymes and blood clotting factors and has no hemolysis at pH 2 [12, 13]. Moreover, the passion fruit active ingredient is a very useful discovery in the pharmaceutical and food industries, since it can be processed in various forms (such as extract, powder, ring, and tablet) and contribute to the reuse of unused resources and environmental preservation. The current study was therefore undertaken to examine new skin functional effects of the pulp and seed extracts of the passion fruit which contain numerous known effective ingredients [6, 18, 19].

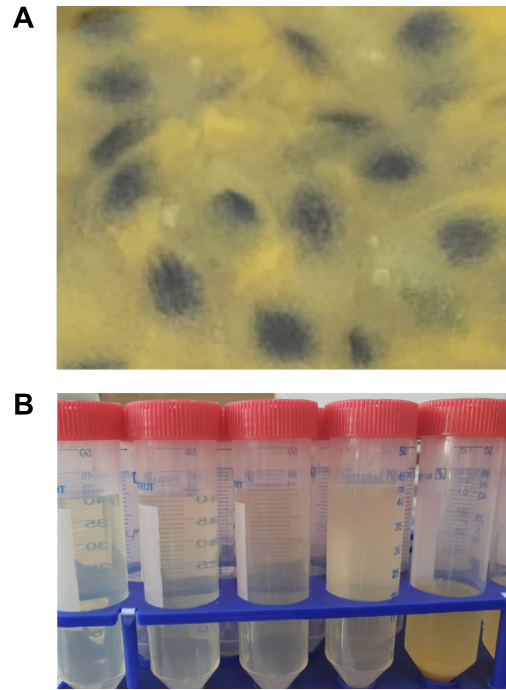

Fig. 1. (A) The pulp, seeds and (B) extracts of the passionfruit fruit were shown.

Materials and Methods

Extraction of passion fruit

Samples of passion fruit pulp and seeds (20 g) were immersed in 200 ml 30% ethanol for 24 hr and active ingredients were extracted at 40 kHz of an ultrasonic tank (Hanshin Tech) for 6 hr. When extracting sample components, if the sample component was extracted using high concentration ethanol, all the fat-soluble components in the sample were extracted, and thus, it may be difficult to mix them with other components, and thus, it was extracted with 30% ethanol. Extracts were then filtered under reduced pressure and subsequently concentrated at 3,000 rpm for 25 min. [2]. The yield of the pulp and seed extracts of passion fruit was 90.4% (Fig. 1).

NO (nitric oxide) anti-inflammatory assay

Cells were seeded at a density of 1×10^5 cells per well in 12-well plates, and cultured in a 37℃ CO_2 incubator for 24 hr. The prepared cells were divided into four groups, as shown in Fig. 2. After 24 hr culture, the media was replaced, and cells were exposed to the passion fruit extract for 1 hr. Inflammation was subsequently induced by treating the cells with 100 ng/ml LPS/well (lipopolysaccharide, stock 1 mg/ml). LPS is an inflammatory agent that activates the RAW 264.7 mouse macrophage cells to produce NO. Stimulated cells were subsequently cultured at 37℃ under CO_2 incubation for 24 hr following which the NO-containing supernatant of each well was extracted, mixed with N1 buffer of NO Plus Detection kit (iNtRON), and reacted at room temperature for 10 min. N2 buffer was added after completion of the 10 min reaction, and the mixture was further incubated for 10 min at room temperature. The amount of NO present in the supernatant was measured at a wavelength of 520 nm using a microspectrophotometer, and the results are presented in Fig. 2 [11, 17].

Levels of NO generation were compared to the NO gen-

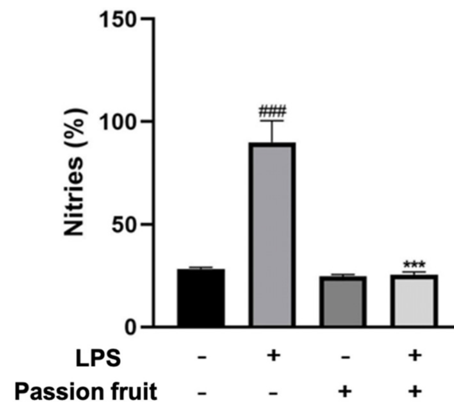

Fig. 2. Diagram confirming the anti-inflammatory effect of passion fruit extract. The experiment was conducted three times.

erated (90.0% average) in the group treated with LPS alone. The average NO generation of LPS-stimulated cells exposed to passion fruit extract was 25.4%, thereby confirming that passion fruit extract exerts an anti-inflammatory effect.

DPPH radical scavenging activity assay

A total of 24 mg DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent was dissolved in 100 ml methanol and stored at -20℃. The stored DPPH solution was adjusted to O.D. 0.98±0.02 before use. Briefly, 1.0 ml DPPH solution was added to the passion fruit sample and reacted in a dark room for 30 min at room temperature. Sample solution was replaced by deionized water in the control group, and trolox was used as the reference compound. Absorbance was measured at 517 nm using the UV-VIS spectrophotometer (Libra S70, Biochrom, UK). DPPH radical scavenging activity (%) of each sample was calculated by the following equation:

Scavenging activity (%) = [(ABS Control – ABS Sample) / ABS Control] ×100

Standard curves were prepared with trolox under the same conditions, and the concentration of trolox exerting similar antioxidant effect as the sample was calculated and marked as µM TE (trolox equivalents)/L sample. The DPPH test results are presented in Table 1 [9].

Cytotoxicity test

A cytotoxicity assay kit (ab211091, Abcam, UK) was used to measure the inhibitory effect of passion fruit on human dermal fibroblast cell proliferation. Cells were treated by serial dilutions of the extract in Iscove's Modified Dulbecco's Medium (IMDM).

Briefly, human-derived fibroblast CCD-986sk cells were cultured at a density of $1×10^4$ cells/well in IMDM cell culture medium at 37℃ and 5% CO_2, and subsequently exposed to serial dilutions (2.5, 5, 10, and 20%) of the sample, followed by incubation for further 24 hr. Cell viability was measured using the Cytotoxicity (MTT) assay kit (Abcam, ab211091, UK), and absorbance was measured at 590 nm using a microplate reader (Synergy H1 Hybrid Multi-Mode Microplate Reader, BioTek, Winooski, VT) [4].

Cell viability (% of control) = (test group O.D.) / (control O.D.) ×10

To verify the effect of drugs on healthy cells, the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used to measure the effects of passion fruit extract on the cell proliferation. $1×10^5$ cells/well (100 µl) were used for B16F10 mouse melanoma cell lines in this assay. All cells were allowed to attach for 24 hr after plating. The next day media was changed and passion fruit extract was added. The cells were then treated with passion fruit extract at increasing concentrations for 24 hr. After the incubation, 100 µl of 5 mg/ml MTT was added to each culture well, and the cells were incubated for 90 min at 37℃. The optical density (OD) of each well at 540 nm was measured by using the Microplate reader (Molecular Devices, USA). Experiments were performed at least three times with representative data presented.

Table 1. The antioxidant effect of the passion fruit extract through the DPPH antioxidant test

Sample	DPPH assay antioxidant activity	
	Scavenging activity (%)	TEAC µmol/ml sample
Passion fruit	5.11±0.17	8.93±0.12

Skin irritation test for passion fruit

The primary irritation human body application test by skin blisters was performed at the KC Skin Clinical Research Center. The skin irritation index was obtained as 0.00, which determines the sample to be a non-irritating product, according to the judgment criteria.

Human Pro-Collagen Ⅰα1 (COL1A1) ELISA assay

The effect of passion fruit on human pro-collagen I alpha 1 gene expression was evaluated in the CCD-986sk cell line. Briefly, $1×10^4$ cells/well were plated and allowed to attach for 24 hr, after which fresh media was replenished the next day. The cells were then treated with passion fruit extract for 24 hr, following which the cells were subjected to ELISA (DY6220-05, R&D Systems, USA). The levels of human pro-collagen I alpha 1 were subsequently monitored by determining the absorbance at 450 nm using a microplate reader (Synergy H1 Hybrid Multi-Mode Microplate Reader, BioTek, Winooski, VT).

Melanin synthesis suppression assay

Samples were serially diluted in Dulbecco's Modified Eagle's Medium DMEM (DMEM) cell culture solution. The mouse melanoma cell line (B16F10) was used in the melanin synthesis inhibition test and was procured from ATCC (American Type Culture Collection). Briefly, $1×10^4$ B16F10 cells/well were cultured with DMEM cell culture medium

at 37℃ in a 5% CO_2 atmosphere. Cultured cells were treated with serially diluted concentrations of 5, 10, and 20% sample extracts and 1 mM arbutin, with simultaneous exposure to 100 nM α-Melanocyte Stimulating Hormone (α-MSH, Sigma-Aldrich, St. Louis, MO, USA). Incubation was continued for further 48 hr. Subsequently, 1N NaOH was added to dissolve melanin in the cells, and absorbance was measured at 405 nm using a microplate reader (Synergy H1 Hybrid Multi-Mode Microplate Reader, BioTek, Winooski, VT) [10].

Melanin content (% of control) = (test group O.D.) / (positive control O.D.) ×100

Result and Discussion

Passion fruit pulp and seeds were extracted with ethanol, filtered, and concentrated for 25 min. The yield of the pulp and seed extracts of passion fruit was 90.4% (Fig. 1) and the pH was 6.7.

Nitric oxide (NO) is generated by inducible nitric oxide synthase (iNOS) and is involved in inflammatory responses. To examine the effect of passion fruit (pH 4.03) on LPS-induced NO production, RAW264.7 cells were treated with the passion fruit extract for 1 hr, and significant inhibition of LPS-induced NO production was observed. As presented in Fig. 2, post-treatment NO generation was compared to levels obtained in the control group (90.0% average) treated with LPS alone. The average NO generation in cells exposed to passion fruit extract and LPS was 25.4%, thereby confirming the anti-inflammatory effect of the extract and indicating that passion fruit suppresses LPS-induced inflammatory responses by inhibiting NO production.

Superoxide radical ($O_2^{\bullet -}$) is a major biological source of reactive oxygen species (ROS). Although superoxide radical is a weak oxidant, it generates powerful and dangerous hydroxyl radicals ($^{\bullet}OH$) as well as singlet oxygen ($^1O_2$), both of which contribute to oxidative stress. The radical scavenging ability of samples is expressed as TEAC μmol/ml sample. In the current study, the DPPH radical scavenging activity assay showed that the scavenging activity of passion fruit was 5.11±0.17% ABTS and 8.93±0.12 μmol TEAC/ml, thereby confirming excellent antioxidant effect of the passion fruit extract (Table 1).

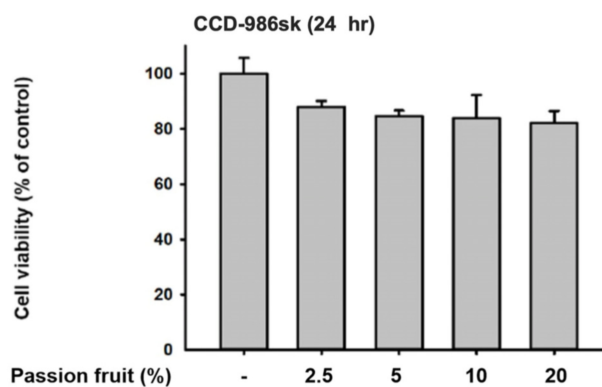

Fig. 3. Illustration confirming the cytotoxicity of passion fruit extract was determined through MTT analysis. Independent 3 repetitions were conducted.

Results of the cytotoxicity assay are presented in Table 2. The CCD-986sk cell line was used to test the inhibitory effect of passion fruit extract on cell proliferation, by exposing the cell-line to 4-fold serial dilution concentrations. Cell viability of the fibroblasts was determined to be 87.95±2.17, 84.54±2.13, 83.76±8.47, and 82.10±4.43%, after treatment with 2.5, 5, 10, and 20% concentrations of the extract, respectively. These results confirm that the passion fruit extract does not exert any cytotoxicity, wherein a compound is deemed to be cytotoxic when the cell survival rate is reduced by more than 30% (based on ISO 10993-5: 2009) (Fig. 3). B16F10 mouse melanoma cell lines were used to test the cytotoxicity effect of passion fruit extract on cell proliferation. When the passion fruit extract was incubated with cells for 24 hr, no significant effect on cell viability was observed in B16F10 (Fig. 4).

The skin irritation index was determined to be 0.00 by the primary irritation human body application test by skin blisters (performed at the KC Skin Clinical Research Center), thereby validating the extract as a non-irritating product according to the judgment criteria (Table 3).

The synthesis of type I collagen, a major component of the skin, is known to be modulated in aging and in various skin diseases and treatments. The human pro-collagen I alpha 1 activity assay showed that exposure to the passion fruit extract decreases the synthesis of procollagen. The

Table 2. Cytotoxicity test

Passion fruit (%)	-	2.5	5	10	20
Cell viability (% of control) toxicity	100.01±5.75	87.95±2.13 None	84.54±2.13 None	83.76±8.47 None	82.10±4.43 None

Table 3. The skin irritation test for passion fruit extract

Result of the skin stimulation test										
	Grade and the number of stimulation decisions (person)								Skin reaction	Skin Irritation index
	1 hr				24 hr					
	1	2	3	4	1	2	3	4		
Passion fruit	-	-	-	-	-	-	-	-	0.00	0.00

Table 4. Human Pro-Collagen Ⅰ α1 (COL1A1) ELISA assay

Passion fruit (%)	-	5	10	20
COL1A1 (ng/ml)	8.10±0.86	7.11±0.71	6.73±0.28	7.16±0.04

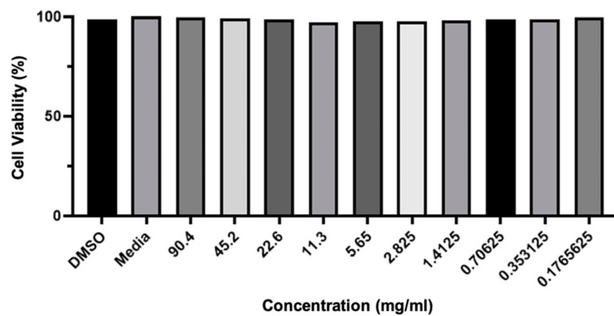

Fig. 4. Effects of passion fruit extract on cell viability in B16F10 mouse melanoma cells.

COL1A1 contents obtained were 7.11±0.71, 6.73±0.28, and 7.16±0.04 ng/ml at varying doses of 5, 10, and 20%, respectively (Table 4 and Fig. 5). Passion fruit extract may help anti-aging skin by reducing procollagen synthesis.

The melanin synthesis inhibition test results are presented in Table 5 and Fig. 6. To compare the amount of melanin produced during culture, B16F10 cells were exposed to passion fruit extract and arbutin, after which the melanogenic stimulating hormone (MSH) was treated. It is judged that there is significance when the corresponding sample t test $p<0.05$. Under normal conditions, melanin is essential for protecting the human skin against radiation, but excess production and accumulation of melanin induces hyperpigmentation, resulting in unwanted afflictions such as melasma, freckles, ephelides, and senile lentigines. Results of the melanin inhibition assay showed that exposure to passion fruit extract significantly suppresses the α-MSH-induced melanin formation. The melanin contents were determined to be 55.12±8.40, 46.52±2.03, and 30.33±2.32%, after treatment with 5, 10, and 20% extracts, respectively (Table 5). These results indicate that the melanin production inhibitory effect of the passion fruit extract is superior to arbutin.

The active ingredient of pulp and seeds of passion fruit

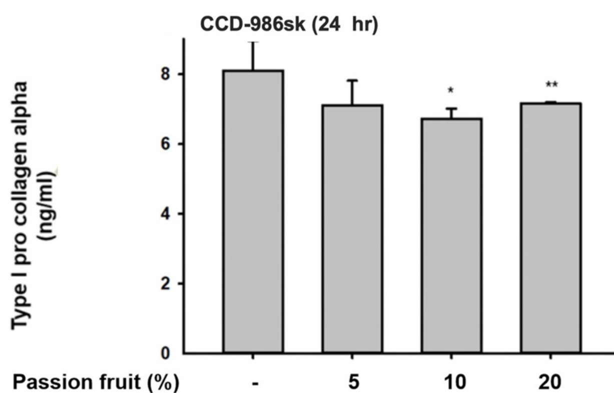

Fig. 5. Human Pro-Collagen Ⅰ α1 (COL1A1) ELISA assay. Independent 2 repetitions were conducted.

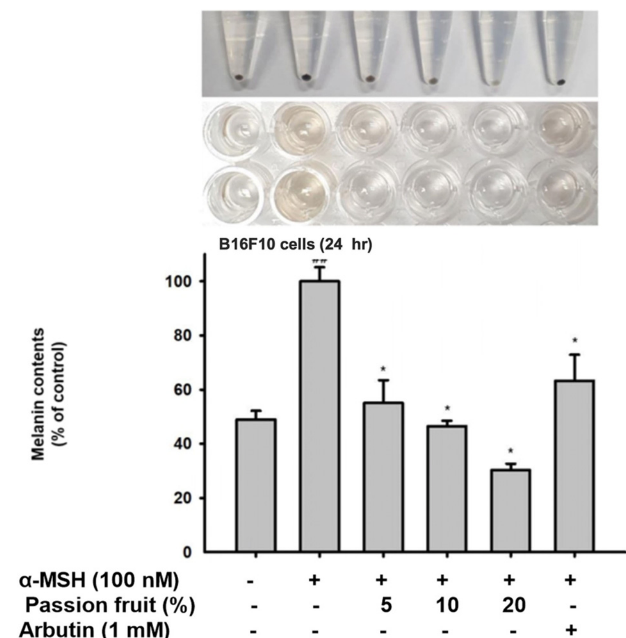

Fig. 6. Diagram confirming the whitening effect of passion fruit extract as determined by the melanin synthesis inhibition test. Independent 2 repetitions were conducted.

Table 5. Melanin inhibition assay

Passion fruit (%)	-	-	5	10	20	-
Arbutin (1 mM)	-	-	-	-	-	+
α-MSH (100 nM)	-	+	+	+	+	+
Melanin (% of control)	48.98±3.19	100.01±5.22	55.12±8.40	46.52±2.03	30.33±2.32	63.32±9.56

which impart skin improvement effects in cosmetic compositions, was obtained by ultrasonic low temperature extraction using ethanol. Comparison of the amount of melanin production in cells was achieved by exposure to the melanin-producing stimulating hormone α-MSH, after treatment with the passion fruit extract and arbutin in the whitening test B16F10 cell line. Inhibition of melanin production was observed to be greater after exposure to passion fruit extract, as compared to arbutin.

The present study examines new skin functional effects of a passion fruit extract. Extracts were prepared using the pulp and seeds of passion fruit. Our results confirm non-toxicity in cells and excellent anti-inflammatory, antioxidant, anti-aging, and whitening effects of the extracts. We propose that the passion fruit extract used in the present study has the potential to be diversely applied in the field of cosmetics and food.

Acknowledgement

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The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

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초록 : 패션 프룻 추출물이 피부에 미치는 새로운 기능적 효과

정미숙[1,3†] · 김순래[2†] · 한창우[1†] · 김현진[1,3†] · 장세복[1*]
([1]부산대학교 자연과학대학 분자생물학과, [2]부경대학교 수산과학대학 식품영양학과, [3]금정제약 주식회사)

본 연구는 패션 프룻(passion Fruit) 열매의 과육과 씨 추출물의 항염증, 항산화, 항노화 및 피부 미백의 기능성 효과에 관한 것이다. 패션 프룻 열매의 추출물은 피부 첩포에 의한 일차 자극 인체적용시험 결과 피부 자극 지수는 0.00으로 판정되었다. 또한 염증매개물질 LPS (Lipopolysaccharides)를 처리하여 Raw 264.7 대식세포를 활성화시켜 NO를 유발하고 패션 프룻 열매 추출물과 반응시켰을 때 LPS만 처리한 그룹의 NO 발생량 보다 현저히 낮은 NO 발생량을 보임으로써 패션 프룻 열매 추출물의 우수한 항염증 효과를 확인할 수 있었다. 그리고 DPPH (2,2-diphenyl-1-picrylhydrazyl) 시험을 통해 패션 프룻 열매 추출물이 5.11%의 ABTS 라디칼 소거능을 보이면서 항산화 효과가 있음을 확인하였다. 피부의 콜라겐 I 형의 합성은 노화와 다양한 피부 질병의 척도인데 인간의 프로콜라겐 I 알파 1 활성도 분석을 통하여 패션 프룻은 프로콜라겐의 합성을 약간 감소시킴을 확인하였다. 또한 멜라닌 억제 시험을 통하여 패션 프룻 열매 추출물의 미백 효능을 확인하였는데 대조군으로 식약처에서 고시한 미백 기능성 성분인 알부틴(arbutin)을 2% 이상 배합한 시료는 월귤나무에서 얻은 히드로 퀴논을 반응시켜 얻은 유기화합물로 일반적으로 백색 분말의 형태로 존재하며 멜라닌 생성을 촉진하는 티로시나아제를 억제하는 효능 효과가 있어 미백 개선의 기능성 성분으로 사용된다. 그러므로 이러한 자연에서 얻은 패션 프룻 열매 추출물의 미백 기능성 효능 확인은 화장품 및 식품의 기능성 원료의 개발에 기여할 수 있을 것으로 기대된다.