Article

Anti-Aging Effects of *Terminalia bellirica*, *Phyllanthus emblica*, *Triphala*, and *Carica papaya* Extracts for Sustainable Youth

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Abstract: As the human lifespan becomes longer, many people invest time and money in managing external beauty. However, managing external beauty has the disadvantage of causing side effects or that the effect does not last. Therefore, research and development are required to maximize effectiveness, eco-friendliness, and sustainably in beauty management. The purpose of this study was to experimentally identify the anti-aging effects, such as skin wrinkle and elasticity improvement, of extracts from *Bahera*, *Phyllanthus emblica*, *Triphala*, and *Carica papaya*, and to confirm their development as whitening and wrinkle functional cosmetic materials. In this study, a solid mixture was prepared using eco-friendly *Terminalia bellirica*, *amla* (*Phyllanthus emblica*), *Triphala*, and *Carica papaya*, and experimental samples were extracted. Antioxidant tests, antibacterial activity tests, polyphenol and flavonoid content, and deodorization tests were conducted to test the efficacy of experimental samples. The procedures and methods of these experiments are summarized in the following article. In this study, we found that the *Bahera*, *Phyllanthus emblica*, *Triphala*, and *Carica papaya* extracts had significant effects on whitening and wrinkle improvement, and that the effects of using ethanol-based extracts as the co-solvent were even greater. In other words, extracts of *Bahera*, *Phyllanthus emblica*, *Triphala* and *Carica papaya* showed antioxidant, whitening, and anti-wrinkle effects, and extracts that used ethanol as a co-solvent showed greater effects. In particular, we found that the optimal concentration of ethanol as a co-solvent maximizes its effectiveness at 70%.

Keywords: anti-aging effect; *Terminalia bellirica*; *amla*; *Phyllanthus emblica*; *Triphala*; *Carica papaya*; eco-friendly materials; sustainable beauty care

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1. Introduction

Rapid industrialization and urbanization are causing serious global environmental pollution and resource depletion, threatening the future of mankind. Recognizing the exhaustion and finiteness of these resources, research on sustainability has recently been actively conducted in various fields, and various alternatives for achieving eco-friendly growth are suggested [1]. In the cosmetics industry, efforts are being made to develop products using natural resources or to substitute sustainable raw materials [2]. In particular, consumer needs for natural cosmetics are leading to the development of new products that promote eco-friendliness.

Due to the development of medical technology and the improvement of living standards, interest in improving skin wrinkles, elasticity, skin whitening, and the related cosmetics market is also expanding [1]. Skin consists of epidermis, dermis, and subcutaneous tissue to protect the body from harmful external factors such as temperature, humidity, and ultraviolet rays [2]. As skin ages or is exposed to ultraviolet rays, collagen synthesis decreases due to the action of fibroblasts and the decrease in the number of cells. In addition, collagenase and elastase, which break down collagen, increase skin moisture loss and decrease skin flexibility and elasticity [3].

Ultraviolet rays are one of the most important environmental factors that cause skin aging [4]. When skin is exposed to ultraviolet light, a harmful metabolism is activated in the skin, causing abnormal cross-linking with collagen and elastin, which causes skin tissue

damage and skin wrinkles. Thus, substances with activity that can inhibit collagenase and elastase may have a skin wrinkle improvement effect [5].

Terminalia bellirica is a deciduous tree of the family *Terminalia* that has an antiviral effect on bacteria and a variety of diseases. Therefore, many studies have been conducted on the antibacterial activity of *Terminalia bellirica*, mainly in *E. coli*, and yellow staphylococcus [6–10]. However, studies on *Terminalia bellirica* in relation to skin wrinkle improvement or elasticity improvement effects are limited. *Phyllanthus emblica* L., Indian gooseberry or *amla*, is known as the “fruit of rejuvenation” and has the effect of preventing various diseases and aging, is essential for beauty and health, and contains a large amount of vitamin C and polyphenols to prevent cell oxidation and reduce free radicals [11]. The antioxidant function of vitamin C prevents cells from being destroyed from excess free radicals, inducing the secretion of insulin-like growth factor-1 (IGF-1), which promotes skin improvement, and inhibiting the secretion of factors such as DKK-1 and TGF-11, thus helping the skin stay healthy [12,13].

Triphala is a combination of three medicinal plants, *Amalaki Phyllanthus emblica* (syn. *Embllica officinalis*) *Phyllanthaceae* family, *Haritaki (Terminalia chebula)* *Combretaceae* family, and *Bahera (Terminalia bellirica)* *Combretaceae* family, and has been extensively used in Ayurveda since ancient times. It is a very useful tool for improving the body's immunity, as it readily promotes the body's ability to form antibodies in order to fight any invasion of antigens [14]. *Amalaki* is an excellent source of vitamin C and also contains carotene, nicotinic acid, D-glucose, D-fructose, riboflavin, empicol, and mucic and phyllemblic acids. *Haritaki* is used in traditional medicine due to the wide spectrum of pharmacological activities associated with the biologically active chemicals present in this plant. It contains anthraquinone glycoside, chebulinic acid, tannic acid, terchebin, vitamin C, and arachidonic, linoleic, oleic, palmitic, and stearic acids. It inhibits the rate of cell proliferation and cell death in cancer cell lines. *Bahera* contains chebulagic acid, ellagic acid and its ethyl ester, gallic acid, fructose, galactose, glucose, mannitol, and rhamnose [15].

According to a study on antibacterial extracts of *Carica papaya* [16], papaya suppresses pathogenic microorganisms such as salmonella and typhoid, which can be used as biochemical indicators for heat treatment processes [17], which is effective in reducing blood pressure and heart rate.

On the other hand, many studies have been conducted on phytotherapy methods, which do not separate specific ingredients of plant extracts but use scientific approaches to separate and refine certain ingredients of plant extracts. In particular, *Terminalia bellirica*, *amla (Phyllanthus emblica)*, *Triphala*, and *Carica papaya* are materials with proven pharmacological effects, so it would be more meaningful to verify the combination of their mixtures rather than the pharmacological efficacy of certain ingredients individually.

Therefore, this study investigated whether extracts of eco-friendly *Terminalia bellirica*, *amla (Phyllanthus emblica)*, *Triphala*, and *Carica papaya* mixtures are likely to be developed as drugs from a sustainable point of view, not in the short term.

2. Materials and Methods

In this study, we manufactured a mixture of solid phases using *Terminalia bellirica*, *amla (Phyllanthus emblica)*, *Triphala*, and *Carica papaya* and extracted experimental samples. Antioxidant tests, antibacterial activity tests, polyphenol and flavonoid content, and deodorization tests were conducted to test the efficacy of the experimental samples. The procedures and methods of these experiments are described in the following sections.

2.1. Manufacture of a Mixture of *Terminalia bellirica*, *Phyllanthus emblica*, *Tripala*, and *Carica papaya*

After cleaning the *Terminalia bellirica*, *Phyllanthus emblica*, *Triphala*, and *Carica papaya* supplied from Jibio Pharm Co., Ltd. (Goyang-si, Korea), the samples were dried at 70 °C for 48 h and ground to a size of 2 mm or less. The ground raw materials were mixed with a certain weight (100 g: 100 g: 100 g: 100 g).

2.2. Manufacturing Test Samples

To prepare the test samples, the supercritical fluid supplied to the extractor (SC-CO₂ extraction system, Ilshin Autoclave Co., Ltd., Daejeon, Korea) for two hours was supplied at a flow rate of about 40 mL/min while maintaining the mixture at 45 to 55 °C, and 100 to 200 bar. The extraction process was carried out four times by contacting the filled solid-state building and extracting the extract from the solid-state building. At this time, a test sample was manufactured in accordance with the conditions of ethanol supply to the extractor.

First, no ethanol was supplied to TATP-1, and 100% ethanol was supplied to TATP-2 at a flow rate of 1.0 mL/min, and 70% ethanol was supplied to TATP-3 at a flow rate of 1.0 mL/min.

Second, the mixture of supercritical fluid and extract was released out of the extractor, deflated to about 50 bar via a pressure regulator (a back pressure regulator 2), and then insulated and expanded to the separator. The extracted extract and fluid were separated from the separator, and the separated fluid was liquefied through a cooler adjusted to −1 °C and stored in a reservoir for reuse. In addition to the fluid circulated and supplied, the fluid stored in the reservoir was supplemented externally to compensate for the loss of fluid from the entire process, and the fluid was pressurized through a pump into a supercritical state and circulated back to the extractor via a heat exchanger. Extracts separated from the separator were filtered with a 0.45 µm membrane filter and concentrated at vacuum and room temperature for 3 h to produce test samples (see Table 1).

Table 1. Test samples.

TATP-1	TATP-2	TATP-3
No ethanol supply	100% ethanol supplied at a flow rate of 1.0 mL/min	70% ethanol supplied at a flow rate of 1.0 mL/min

2.3. Experiments on Total Polyphenols and Total Flavonoid Content

1. Total polyphenols experiment

First, 100 mg of each of the three prepared samples was taken and diluted to 100 mL using 80% ethanol. After taking 100 mg of gallic acid, 80% ethanol was used to make 100 mL. Second, amounts of 0.1, 0.2, 0.5, and 1.0 mL of this solution were taken, and a solution diluted to 5 mL was used as the standard solution. After adding 100 µL of the solution and 100 µL of sodium carbonate to an e-tube, 100 µL of Folin-Ciocalteu reagent (Sigma, St. Louis, MO, USA) was added, mixed with the vortex for 30 s, and left in a dark place for 30 min. The absorbance value of the reaction solution was measured using a UV-vis spectrophotometer (Bekman, Germany) at 750 nm.

2. Flavonoid experiment

First, 100 mg of each of the three prepared samples was taken and diluted to 100 mL using 80% ethanol. After taking 100 mg of quercetin separately, 80% ethanol was used to make 100 mL. Second, amounts of 0.1, 0.2, 0.5, and 1.0 mL of this solution were taken, and a solution diluted to 5 mL was used as the standard solution. In total, 500 µL of test liquid and standard liquid were added to an e-tube with 100 µL of 10% aluminum nitrate and 100 µL of 1 M potassium acetate. After 40 min of mixing, the absorbance at 415 nm was measured using a UV-vis spectrophotometer.

2.4. Antioxidant Experiment

1. ABTS radical scavenging activity

After taking 100 mg of each of the three prepared samples, water was added and diluted to 100 mL. A mixture of 7 mM ABTS (Sigma, USA) and 2.45 mM potassium persulfate was reacted for 12 h at room temperature in a dark place to form an ABTS cation. It was then adjusted by adding ethanol at 734 nm so that the absorbance value was 0.70 ± 0.02 . Amounts of 100 µL of the test solution and 100 µL of the prepared ABTS

solution were added to 96-well plates to react at room temperature for 7 min and measured using a microplate reader (Epoch™2, BioTECH, Winooski, VT, USA) at 734 nm. The ABTS radical elimination rate, that is, ABTS radical scavenging activity, was calculated as a percentage (%) compared to the test solution.

2. DPPH radical scavenging activity

After taking 100 mg of each of the three prepared samples, water was added and diluted to 100 mL. Then 100 µL of the test fluid and 100 µL of 0.2 mM DPPH (Sigma, NY, USA) were put in 96-well plates and, after 30 min, absorbance was measured at 517 nm using a microplate reader. The DPPH radical elimination rate, that is, DPPH radical scavenging activity, was calculated as a percentage (%) compared to the test solution.

3. SOS-like Activity

The three prepared samples were diluted in water at a constant concentration, and then used as a sample. An amount of 2.6 mL of tris-HCl buffer corrected at 8.5 mL and 0.2 mL of 7.2 mM pyrogallol were added to 0.2 mL of the test solution and reacted at 25 °C for 10 min. Then 0.1 mL of 1 N HCl was added to the reaction solution to stop it. The amount of pyrogallol (Sigma, NY, USA) oxidized was measured at 420 nm for absorbance.

4. Xanthine oxidase inhibitory activity

Three prepared samples were diluted in water at a certain concentration, and then used as a sample. Then 0.6 mL of 0.1 M potassium phosphate buffer (pH 7.5) and 0.2 mL of 1 mM xanthine were added to 1.0 mL of the test solution. Then 0.1 mL of 0.2 U/mL xanthine oxidase was added to stop the reaction. The produced uric acid was measured for absorbance at 292 nm.

2.5. Whitening Activity Experiment

Three prepared samples were diluted in water at a certain concentration, and then used as a sample. An amount of 0.5 mL of 175 mM sodium phosphate buffer (pH 6.8) was added to 0.1 mL of the test solution and 0.2 mL of 10 mM of L-DOPA (3,4-dihydroxy-L-phenyl-alanine) was also added to 0.1 mL of the test solution. Then 0.2 mL of a 110 U/mL solution was added to react at 25 °C for 2 min, and the produced DOPA chrome was measured for absorbance at 475 nm.

2.6. Anti-wrinkle Evaluation Experiment

Collagenase inhibitory activity and elastase inhibitory activity experiments were performed for an anti-wrinkle evaluation.

1. Collagenase inhibitory activity

Three prepared samples were diluted in water at a certain concentration, and then used as a sample. Then 4 mM calcium chloride was added to 0.1 M tris-HCl buffer (pH 7.5) and 0.2 mL of the solution was dissolved in 4-phenylazo benzyl oxycarbonyl-Pro-Leu-Gly-Pro-D-Arg (0.3 mg/mL). Then 0.3 mL of 200 U/mL collagenase type I (Sigma, NY, USA) was added to react at room temperature for 20 min. To stop the reaction, 0.5 mL of 5% citric acid was added and 1 mL of ethyl acetate was added to measure absorbance at 320 nm.

2. Elastase inhibitory activity

Three prepared samples were diluted in water at a certain concentration, and then used as a sample. After adding 50 µg/mL of the pancreatic solution, N-succinyl- (LA) 3-p-nitroanilide (1 mg/mL) dissolved in 50 mM tris-HCl buffer (pH 8.6) was added to react for 30 min and absorbance was measured at 410 nm.

2.7. Cell Stability Experiment

A typical cytotoxicity test, MTT assay (Sigma, USA), was used to evaluate the stability of the samples. The quantity was measured by modifying the Mosmann method. HaCaT cells were busy 1×10^4 cells/mL, incubated for 24 h, then replaced with a new medium

containing samples diluted at concentrations of 0.5, 1.0, 1.5, and 2.0 mg/mL. Then 20 µL of EZ-Cytox per well was added, and absorbance was measured with an ELISA reader at 450 nm after incubation at 37 °C, with a 5% CO₂ incubator. Cell viability was calculated using the following Equation (1):

$$\text{Cell viability (\%)} = [(\text{Exp.} - \text{Blank}) / \text{Control}] \times 100 \quad (1)$$

Exp.: Absorbance of extracts containing cells.

Blank: Absorbance of extracts that do not contain cells.

Control: Absorbance of distilled water including cells.

3. Results

3.1. Total Polyphenols and Total Flavonoid Content

The polyphenol content of TATP-3 was measured at 195.7 mgGAE/g, showing the highest content among the three samples. For TATP-1 without co-solvent for supercritical fluids, the polyphenol content was measured at 95.2 mgAE/g, and for TATP-2 with 100% ethanol, the polyphenol content was measured at 143.8 mgAE/g. These analysis results confirm that the content of polyphenols increases when the appropriate concentration of co-solvent is used for supercritical fluids.

In addition, the flavonoid content of TATP-3 was measured at 97.7 mgQE/g, showing the highest content among the three samples. The flavonoid content of TATP-1 without co-solvent for supercritical fluids was measured at 42.4 mgQE/g, and the flavonoid content of TATP-2 with 100% ethanol as a co-solvent was measured at 54.1 mgQE/g. The results of the flavonoid content experiment also showed the same tendency as the polyphenol content (see Figure 1).

Figure 1. Total polyphenols and total flavonoid content of the samples.

3.2. Anti-Oxidation

DPPH radical analysis of TATP-3 showed 68.3% at concentrations of 2.0 mg/mL, the highest antioxidant content among the three samples (see Figure 2a). On the other hand, TATP-2 with no co-solvent used in supercritical fluids showed 53.7% antioxidant content at 2.0 mg/mL concentration and 61.3% at 2.0 mg/mL concentration using a co-solvent of 100% ethanol. All experimental materials were analyzed for their scavenging activity as concentration dependencies, and all were found to be lower than the control group ascorbic acid. In addition, ABTS radical analysis for TATP-3 found the highest concentration of 84.9% at 2.0 mg/mL concentration, while TATP-1 found 57.9% at 2.0 mg/mL concentration without co-solvent, and TATP-2 with 100% ethanol as co-solvent found 64.7% at 2.0 mg/mL concentration (see Figure 2b). These experimental results showed the same tendency as the experimental results of DPPH (see Figure 2).

Figure 2. Radical scavenging activity of the samples.

As shown in Table 2, SOS-like activity analysis of TATP-3 showed the highest activity at 38.8% at 2.0 mg/mL concentrations. On the other hand, TATP-2 with no co-solvent in supercritical fluids showed 27.5% activity at a concentration of 2.0 mg/mL, and TATP-2 with a co-solvent of 100% ethanol showed poor activity at 35.6% at a concentration of 2.0 mg/mL. All experimental materials were analyzed for their scavenging activity due to concentration dependence, and all were found to be lower than the control group ascorbic acid. For TATP-3, the xanthine oxidase inhibitory analysis found that the highest concentration was 41.3%; while for TATP-1 without co-solvent for supercritical fluids, it was found to be 33.6% at 2.0 mg/mL concentration and 100% ethanol as co-solvent.

Table 2. SOS-like activity and xanthine oxidase inhibition activity.

Extract	SOD-Like Activity (%)				Xanthine Oxidase Inhibition (%)			
	0.5 mg/mL	1.0 mg/mL	1.5 mg/mL	2.0 mg/mL	0.5 mg/mL	1.0 mg/mL	1.5 mg/mL	2.0 mg/mL
Control	95.4 ± 7.5	95.0 ± 9.7	96.3 ± 2.6	98.8 ± 1.6	96.3 ± 8.2	96.9 ± 4.8	98.7 ± 1.0	99.6 ± 1.5
TATP 1	11.5 ± 1.1	15.0 ± 3.8	21.5 ± 4.4	27.5 ± 3.7	15.0 ± 2.9	21.6 ± 1.4	29.4 ± 2.8	33.6 ± 4.5
TATP 2	17.0 ± 3.0	23.6 ± 4.5	25.1 ± 3.4	35.6 ± 3.8	21.1 ± 1.1	29.3 ± 0.7	33.6 ± 5.9	35.1 ± 3.6
TATP 3	24.5 ± 1.9	27.0 ± 2.5	33.2 ± 3.2	38.8 ± 7.7	29.4 ± 1.3	36.9 ± 1.8	36.3 ± 4.4	41.3 ± 3.1

3.3. Whitening Activity

Tyrosinase inhibitory activity analysis showed that TATP-3 has the highest inhibitory activity of 33.7% at 2.0 mg/mL concentrations. On the other hand, TATP-1 with no co-solvent in supercritical fluids showed 23.2% activity at 2.0 mg/mL concentrations, and TATP-2 with 100% ethanol was found to show poor inhibitory activity compared to TATP-3 at 2.0 mg/mL concentrations. All experimental materials were analyzed for their elimination activity due to concentration dependence, and they were all found to be lower than the control group ascorbic acid (see Figure 3).

Figure 3. Tyrosinase inhibitory activity of the samples.

3.4. Anti-Wrinkle Evaluation

Collagenase inhibitory activity and elastase inhibitory activity experiments were conducted for an anti-wrinkle evaluation, and the results are shown in Table 3. The collagenase inhibitory activity analysis of TATP-3 showed the highest inhibitory activity at 58.1% at 2.0 mg/mL concentrations. By comparison, TATP-1 without co-solvent in supercritical fluids showed 41.3% collagenase inhibitory activity at 2.0 mg/mL concentrations and 53.3% collagenase inhibitory activity at TATP-2 with co-solvent concentrations. All experimental materials were analyzed for their elimination activity with concentration dependence, and they were all found to be lower than the control group ascorbic acid.

Table 3. Collagenase inhibition activity and elastase inhibition activity.

Extract	Collagenase Inhibition (%)				Elastase Inhibition (%)			
	0.5 mg/mL	1.0 mg/mL	1.5 mg/mL	2.0 mg/mL	0.5 mg/mL	1.0 mg/mL	1.5 mg/mL	2.0 mg/mL
Control	42.7 ± 1.3	64.5 ± 1.7	72.0 ± 1.8	86.1 ± 1.4	53.6 ± 1.5	62.7 ± 0.7	78.2 ± 0.4	82.4 ± 0.7
TATP 1	18.0 ± 0.8	23.4 ± 1.4	34.6 ± 1.3	41.3 ± 0.9	13.2 ± 0.8	27.8 ± 0.5	31.6 ± 0.9	41.4 ± 1.2
TATP 2	24.5 ± 1.1	31.0 ± 0.6	44.3 ± 0.5	53.3 ± 0.8	18.8 ± 1.1	31.9 ± 0.8	38.9 ± 2.0	42.9 ± 1.3
TATP 3	28.5 ± 1.9	37.0 ± 1.9	49.5 ± 2.0	58.1 ± 0.4	21.0 ± 1.6	36.0 ± 1.5	41.8 ± 1.7	48.6 ± 0.8

Meanwhile, analysis of elastase inhibitory activity in TATP-3 showed 48.6%, the highest concentration at 2.0 mg/mL. On the other hand, the elastase inhibitory activity of TATP-1 without co-solvent was measured at 41.4% at concentrations of 2.0 mg/mL, and the elastase inhibitory activity of TATP-2 with 100% ethanol as co-solvent was analyzed at concentrations 2.0 mg/mL. Thus, the results of the elastase inhibitory activity analysis showed the same tendency as the collagenase inhibitory activity analysis results.

3.5. Cell Stability

The cytotoxicity of the extracts in this study was tested at 0.5, 1.0, 1.5, and 2.0 mg/g based on the cell viability (100%) of the untreated group, showing no cytotoxicity for all samples at all concentrations. Thus, the stability of TATP-3 could be confirmed in the HaCaT cells (see Figure 4).

Figure 4. HaCaT cell viability.

4. Discussion and Conclusions

As the human lifespan increases, modern people aim to live a happy life with anti-aging measures, such as improving skin wrinkles and elasticity, due to aging beyond a healthy life, and many studies are being conducted on this. In addition, as consumer needs diversify, the preference for eco-friendly materials over chemical materials is increasing. That is, research on plant extracts with a special performance to maintain sustainable youth has been actively conducted [18]. This study attempted to develop extracts of various plants with the aim of improving skin wrinkles and elasticity to maintain sustainable youth. The purpose of this study was to experimentally identify the anti-aging effects such as skin wrinkle and elasticity improvement of extracts from *Bahera*, *Phyllanthus emblica*, *Triphala*, and *Carica papaya*, and to confirm their development as whitening and wrinkle functional cosmetic materials [19].

As a result of the study, polyphenol and flavonoid compounds were shown to play an important role in whitening and antioxidant activity by inhibiting or removing the generation of free radicals in the body to prevent cell damage [20]. Representative natural antioxidants widely distributed in nature include tocopherols, flavonoids, and polyphenols, and, among them, the total polyphenol content is reported to be a very important factor determining the antioxidant activity of foods [21]. In addition, flavonoids, compounds with a C6-C3-C6 structure, the basic structure of which is a flavone, are contained abundantly in the flowers, stems, and fruits of plants, and are reported to have various functions such as antioxidant, anti-cancer, and anti-inflammatory effects [22]. According to the results of the total polyphenol and total flavonoid experiments, the contents of polyphenols and flavonoids increased when an appropriate concentration of co-solvent was used in the supercritical fluid, and the extract exhibited high antioxidant activity.

DPPH radicals, ABTS radicals, SOS-like activity, and xanthine oxidase inhibitory activity were analyzed to evaluate antioxidant activity, and, according to the results, TATP-3 showed high antioxidant activity. We judged that the antioxidant activity of TATP-3 is due to flavonoids and polyphenol-based components, and the accurate mechanism of the antioxidant activity should be investigated using the standard materials of individual components. According to the results of the antioxidant activity tests, the extracts are judged to be very suitable as natural cosmetics materials.

According to the results of the *Tyrosinase* activity analysis to identify the whitening effect, TATP-3 showed a high inhibitory activity of 33.7% at a concentration of 2.0 mg/mL, and all samples were identified to have lower activity compared to ascorbic acid, which was the control. *Tyrosinase* is an enzyme involved in the initial rate-determining stage, which is the most important stage in the melanin biosynthesis pathway in the human body. If the activity of this enzyme is suppressed, melanin production will be suppressed.

The collagenase and elastase inhibitory activity was analyzed to identify the wrinkle improvement effects, and, according to the results, concentration-dependent scavenging activity was analyzed in all samples, and it was identified that the activity of all samples was lower than that of ascorbic acid, which was the control. Collagen and elastin form network structures in the dermal tissue of the skin to maintain the elasticity of the skin. However, collagen and elastin are broken by collagenase and elastase in their network structure, which is the main cause of wrinkles [23,24]. The extracts used in this experiment effectively inhibited collagenase and elastase.

Causes of skin aging include stress, lack of sleep, exposure to ultraviolet (UV) and undernourishment [18], excluding age-induced natural aging and photo-aging. In addition, reactive oxygen species (ROS), allergy-causing substances and physical stimuli, as well as inflammation, immune abnormalities, epidermal homeostasis imbalances, and other skin diseases also contribute to skin aging [19]. Wrinkles reduce the rate of proliferation of cells taking up the basal cell layer of the epithelium, making the epithelium thinner, and making the skin easily wrinkled [20]. Another way of reducing wrinkles and skin elasticity in skin aging is the reduction of the extracellular matrix (ECM) in the dermis [21]. The extracellular substrate is the site of the substrate responsible for the structural support between the cells and consists of a compositional protein that exhibits various structures and characteristics. Major ingredients include collagen, elastin, proteoglycans, laminin, and fibronectin, among which collagen and elastin account for more than 90% of the protein [22]. Wrinkle generation in the skin can be caused by weakening cell regeneration capabilities in the skin layer due to UV exposure, reduced synthesis of collagen, elastin fiber protein, and a reduced amount of ECM in the dermis [23,24].

Bahera, *Phyllanthus emblica*, *Triphala*, and *Carica papaya* extracts showed strong inhibitory activities for *Tyrosinase* activity. The mechanism of skin whitening by inhibiting *Tyrosinase* activity is similar to that of arbutin, which is already commercially used as a whitening material. In this study, extracts from *Bahera*, *Phyllanthus emblica*, *Triphala*, and *Carica papaya* showed active inhibitory mechanisms of *Tyrosinase*, such as the synthetic chemical arbutin. This mechanism of action is believed to be attributable to the reduction of melanin production by the inhibition of the activity of *Tyrosinase* in skin cells. Furthermore, the analysis of cell survival in up to 2.0 mg/g concentrations of *Bahera*, *Phyllanthus emblica*, *Triphala*, and *Carica papaya* extracts showed no cytotoxicity, and that all samples were highly effective in replacing existing arbutin and safe anti-materials.

In addition, the extracts of *Bahera*, *Phyllanthus emblica*, *Triphala*, and *Carica papaya* were measured for DPPH radical elimination and ABTS radical elimination of functional natural materials. *Bahera*, *Phyllanthus emblica*, *Triphala*, and *Carica papaya* extracts were evaluated for safety on HaCaT cells using a cytotoxicity test, an MTT assay, which showed no cytotoxicity for all samples at concentrations that showed wrinkle improvement. In other words, no cytotoxicity was present up to 2.0 mg/g concentrations.

These results suggest that *Bahera*, *Phyllanthus emblica*, *Triphala* and *Carica papaya* extracts increase collagen and elastin synthesis, potentially inhibiting damage to skin binding cells by aging. However, despite these meaningful research results, this study had limitations in not being able to apply clinical trials or animal test models. Subsequent studies need to be conducted on whitening, and the establishment of functional mechanisms and surface components for whitening and crease improvement extracts for *Bahera*, *Phyllanthus emblica*, *Triphala*, and *Carica papaya*, and animal model experiments, which can lead to the development of a safe natural material for whitening and wrinkle improvement. The plant extract developed in this study can be used as a basic material in the development of safe natural plant materials with complex functionality in improving facial skin to maintain sustainable youth. In particular, this study is meaningful in that it investigated sustainable aging management with eco-friendly natural plants rather than chemical reactions at a time when human health is most important due to the coronavirus. In addition, it is hoped that companies related to this study will be helpful in designing sustainable products using natural resources.

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