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Physiological effects of formulation containing tannase-converted green tea extract on skin care: physical stability, collagenase, elastase, and tyrosinase activities

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ABSTRACT

Background: Green tea contains numerous polyphenols, which have health-promoting effects. The purpose of this study was to evaluate the effect of tannase-converted green tea extract (TGE) formulation on the physical stability and activities of skin-related enzymes.
Methods: Physical stability was evaluated by measuring the pH, precipitation, and colors at 25 ± 2 °C/ambient humidity and at 40 ± 2 °C/70% ± 5% relative humidity for 4 months. Activities of collagenase, elastase, and tyrosinase as skin-related enzymes were assessed on TGE formulation.
Results: The concentrations of epigallocatechin-3-gallate and epicatechin-3-gallate in green tea extract were greatly decreased to the extent of negligible level when treated with tannase. The formulation containing 5% tannase-converted green tea extract showed relatively stable pH, precipitation, and color features for 16 weeks. When TGE was added to the formulation, there was a significant increase in the inhibition of elastase and tyrosinase activities ($p < 0.05$) compared with the formulation containing 5% normal green tea extract.
Conclusion: The TGE could be used in cosmetics as skin antiwrinkling or depigmenting agent.

1. Introduction

Facial appearance is one of the important factors influencing social relations in many situations. People tend to judge their personality based on facial appearance.[1] Cosmeceuticals, which are applied to skin, are widely used to improve such an appearance. Ingredients in cosmeceuticals should penetrate the skin and become systemically available to be effective.[2] Therefore, the research and development for cosmeceuticals should not only include sources, structures, skin-interactive mechanisms of active ingredients, but also their safety and efficacy on the targeted components of skin.[3]

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Plant extracts have been widely used as ingredients of topical agents for wound healing and antiaging. Some of the commonly used plants for this purpose are ginkgo, ginseng, grape seeds, papaya, citrus fruits (e.g., lemon), lavender, rosemary, soy, aloe vera, and green tea, etc.[4] Plant extracts usually contain polyphenols such as flavonoids, which react with reactive oxygen species (ROS) to neutralize free radicals.[5] At present, there is a strong tendency in the cosmetic industry to develop multifunctional cosmetics with high antioxidant activity. According to the oxidative stress theory, the major causes of skin aging are an excessive production of ROS[6] and a reduction of antioxidant activity with age.

Catechins, the polyphenols in green tea, have beneficial effects on human health.[7] In recent years, they have received a great deal of attention due to their potent antioxidant activities,[8] and it was reported that green tea extract can be used to help treat hypercholesterolemia, and protect individuals against disorders caused by the aging process.[9] Moreover, several studies have reported that the catechins in green tea inhibit the activities of collagenase[10] and tyrosinase activity, subsequently improving skin health.[11]

Tannase, also known as tannin acyl hydrolase (EC 3.1.1.20), is a kind of inducible enzyme produced by fungi, yeast, and bacteria. Tannase has mostly been characterized by its hydrolyzing activity on the ester bond (galloyl ester of an alcohol moiety) and the depside bond [galloyl ester of gallic acid (GA)] of substrates such as tannic acid, (–)-epigallocatechin-3-gallate (EGCG), (–)-epicatechin-3-gallate (ECG), and chlorogenic acid. Lu and Chen[12] reported that the tannase-derived bioconversion on catechins compositions in green tea enhanced the scavenging ability on radicals, such as superoxide anions, and hydrogen peroxide. It has also been reported that the antioxidant activities and chelation of metal ions are improved by tannase treatment.[12] However, only limited studies are available on the physical stability and physiological effects of a tannase-converted green tea extract (TGE) formulation.

In this study, we investigated the stability of a TGE formulation and its effect on collagenase, elastase, and tyrosinase activities as major enzyme markers for skin health.

2. Methods

2.1. *Preparation of TGE*

Green tea leaves were obtained from the Hadong area of Korea. The green tea leaves were ground and pulverized in a mortar, and then mixed with distilled water (DW) for 30 minutes in a 10-L reactor (200 g/4 L). The mixture was incubated at 80 °C for 20 minutes, and centrifuged at 3000 *g* for 15 minutes at 5 °C. The clear supernatant [normal green tea extract (NGE)] was used for experiments. The green tea extract (4 L) thus prepared was combined with 1 g of tannase (Visionbiochem, Gyeonggido, Korea) and then incubated in a water bath at 35 °C for 20 minutes followed by centrifuging at 3000 *g* for 15 minutes at 5 °C. The supernatant was used as the TGE for further analyses. The compositions of the base formulation (vehicle), the formulation containing 5% NGE (FNGE), and the formulation containing 5% tannase-converted green tea extract (FTGE) are shown in Table 1.

Table 1 – Compositions of cosmetic formulations.

Compositions (%)	Vehicle	FNGE	FTGE
Deionized water	61.5	56.5	56.5
Carbomer (1% solution)	30	30	30
Hyaluronic acid	5	5	5
Beta glucan	3	3	3
Triethanolamine	0.3	0.3	0.3
Methylparaben	0.1	0.1	0.1
DS-49	0.1	0.1	0.1
NGE	0	5	0
TGE	0	0	5
Total	100	100	100

DS-49, disodium-2,2′-dihydroxy-4,4′-dimethoxy benzophenone sulfonic acid; FNGE, formulation containing 5% normal green tea extract; FTGE, formulation containing 5% tannase-converted green tea extract; NGE, normal green tea extract; TGE, tannase-converted green tea extract; vehicle, base formulation.

2.2. *Physical stability*

The physical stability assay used in this study was based on the method reported by Zhang et al.[13] The samples in impermeable polypropylene containers were stored at 25 ± 2 °C with ambient humidity (AH), and at 40 ± 2 °C with 70% ± 5% relative humidity (RH) for 4 months. The pH, precipitation, and color features of samples were evaluated after storage for 1, 2, 3, 4, 8, 12, and 16 weeks at room temperature. Approximately 1 g of the sample was diluted with distilled water up to a volume of 10 mL. This sample mixture was then homogenized. The pH of the samples was measured using a pH meter (Systronics, Inc., Ahmadabad, India). Precipitation was conducted by measuring the supernatant after centrifuging at 3000 *g* for 30 minutes. The color of samples was measured using a colorimeter (Model CR300; Minolta Camera Co. Ltd. Inc., Osaka, Japan). The colorimeter was calibrated using a Minolta standard-white reflector plate in advance. The data were expressed as *L* (degree of lightness), *a* (degree of redness), and *b* (degree of yellowness) values based on the Hunter color system. The total color difference (Δ*E*) is calculated as follows:

$$\Delta E = \sqrt{(L - L')^2 + (a - a')^2 + (b - b')^2}$$

where *L*, *a*, and *b* are colors of the samples; *L*′, *a*′, and *b*′ are colors of the base at time zero.

2.3. *Analysis of total polyphenol and flavonoid contents*

Total polyphenol contents were examined using the Folin–Ciocalteu method.[14] The reaction mixture includes 0.79 mL of DW, 0.01 mL of sample, and 0.05 mL of Folin–Ciocalteu reagent. After exactly 1 minute, 0.15 mL of 20% sodium carbonate was added to the mixture, followed by standing at room temperature in darkness for 120 minutes. The absorbance was measured at 750 nm, and the total polyphenol content was calculated with GA as the standard. The total flavonoid content was determined by the method