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연구논문

# 가지 열수추출물의 피부생리활성에 관한 연구

김란*

# A Study of Physiological Activities of the Thermal Treated Eggplant on the Skin

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**Abstract:** The purpose of the present research was to investigate the physiological activities of the thermal treated eggplant on the skin. Five minute of thermal treatment at 100ºC had the highest polyphenol content of eggplant. However, below and over 5 min of thermal treatment time, they did not increase. When water and ethanol extracts were used, the maximum DPPH radical scavenging activities were obtained, 66.3 and 62.8%, respectively. Among various extracts, the acetone extract gave the highest cosmetic activity. Especially, when acetone extract (15.0 mg/mL) was used, the maximum inhibition activities of tyrosinase, elastase, and collagenase were obtained, 83.4, 78.2 and 62.5%, respectively. These results suggest the anti-wrinkle and whitening and effects of acetone extract were excellent. Therefore, it is should be considered as a promising candidate for novel cosmetic agents.

**Keywords:** Thermal treatment eggplant, Antioxidant, Tyrosinase, Elastase, Collagenase

## 1. INTRODUCTION

Most vegetables are heated either by boiling, steaming or microwaving before being consumed [1]. Several studies have reported that these heating processes cause many changes in the chemical composition of vegetables. For example, the antioxidant capacity and total phenolic content of pepper (*Capsicum* spp.), green bean (*Phaseolus vulgaris*), broccoli (*Brassica oleracea*), spinach (*Spinacia oleracea*), and sweet corn (Zea mays) increase after boiling, steaming, or microwaving [1,3]. Heating also increased total phenolic content in pungent peppers (*Capsicum annuum*) [4]. In contrast, total phenolic content and antioxidant capacity of tomatoes (*Lycopersicon esculentum*) decrease by boiling, baking, or frying [5].

The antioxidant capacity and total phenolic content of fresh cut broccoli decrease significantly by microwave heating [6]. The antioxidant capacity of garlic (*Allium sativum*) decreases by drying and steaming [7]. Also, the phenolic content was found to decrease in nonpungent peppers (*Capsicum annuum*) by heating [4].

Eggplant (*Solanum melongena* L.) is an agronomically important nontuberous crop belonging to the *Solanaceae* family, which is important for its richness in healthy components and it is also widely consumed in the world. Eggplant contains important phytonutrients such as phenolic compounds which have high antioxidant capacities [8] and anthocyanins [9] like nasunin and delphinidin conjugates [10]. The main polyphenols found in eggplant are phenolic acids such as chlorogenic acid, caffeic acid and p-coumaric acid, but this vegetable is poor in provitamin A and vitamin E. However, the presence of vitamins C and B in this fruit has been established [11]. Studies have shown that eggplant extracts suppress the development of blood vessels required for tumor growth and metastasis [12], and inhibit inflammation that can lead to atherosclerosis [13]. Different sol-

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vent systems have been used for the extraction of polyphenols from plant material. The yield and antioxidant activity of natural extracts is dependent on the solvent used for extraction. Several procedures have been proposed [14]: extraction using fats and oils, organic solvents, aqueous alkaline solutions and supercritical carbon dioxide. Aqueous mixtures of ethanol, methanol and acetone, are commonly used [15]. Wang and Helliwell [16] reported that aqueous ethanol was superior to methanol and acetone for extracting flavonoids from tea. However, in another work, water was found to be better solvent, for extracting tea catechins, than were 80% methanol or 70% ethanol [15]. Nonetheless, there was no information on the antioxidant activity, and cosmetic activity of various extracts of thermal treated eggplant so far.

In this study, firstly, to study chemical composition of raw eggplant, proximate composition, amino acid composition, fatty acid composition, and physicochemical properties were investigated. Secondly, DPPH radical scavenging activity, and the inhibitory activities of tyrosinase, elastase and collagenase using various extracts of thermal treated eggplant were investigated.

## 2. MATERIAL AND METHODS

### 2.1. Sample preparation

Eggplant grown in Gusan at Jeonbuk, South Korea in 2015 were obtained. After thermal treatment of eggplant, Samples (100 g) were extracted with 500 mL of water, ethanol, acetone, and hexane using a soxhlet apparatus at room temperature for 6 hr. Next, the extracts were evaporated. After filtering through Whatman No. 1 filter paper. After standing overnight at 4°C, the mixture was centrifuged and the supernatants were again evaporated. The resulting solid was used as extract. The extracts were used directly for analyses of polyphenol components and stored at 4°C for further use.

### 2.2. Proximate composition

Proximate compositions were determined by the official and tentative methods of the American oil chemists' society [17]. Especially, the protein concentration was determined as percent nitrogen × 6.25 using the micro Kjeldahl technique.

### 2.3. Fatty acid composition

GC-MS analysis was carried out to determine the composition of oil extracted from raw eggplant using hexane. The oil was analyzed on a HP-5MS capillary column (30 m × 0.35 mm × 0.2 m), and GC-2010 (Shimadzu, Japan) coupled with a GC-MS-QP2010plus (Shimadzu, Japan). Oven temperature was increased 50~100°C at 3°C/min, 100~200°C at 2°C/min and then 200~280°C at 5°C/min, with helium as a carrier gas at 1.2 L/min and the injector temperature was 200°C.

### 2.4. Amino acid composition

Sample (0.5 g) was added to 3mL of HCl (6 N) and the hydrolysis was carried out for 24 hr at 121°C. The mixture was then pressure-concentrated and 10 mL of sodium phosphate buffer (pH 7.0) was added. An aliquot of 1 mL of solution was filtered by membrane filter (0.2 mM) and then analyzing by automatic amino acid analyzer (Biochrom 20, England).

### 2.5. Total phenolic content

Total phenolic content were determined with Folin-Ciocalteu reagent with gallic acid as a standard. A 1 mL portion of the sample was mixed with 1 mL of Folin and Ciocalteu's phenol reagent. After 3 min, 1 mL of saturated $Na_2CO_3$ (35%) was added to the mixture, which was brought to a volume of 10 mL by the addition of distilled water. The reaction was maintained in the dark for 90 min, and its absorbance was measured at 725 nm relative to a blank.

### 2.6. DPPH radical scavenging activity

The DPPH (1, 1-diphenyl-2-picry-hydrazil) radical scavenging activity was determined by spectrophotometer. A sample (1 mg/mL) was added to 1 mL of DPPH (10 mM) in methanol. The mixture was shaken and maintained at room temperature for 10 min. The absorbance was measured at 517 nm. DPPH radical scavenging activity was calculated with the following formula:

$$\text{DPPH radical scavenging activity} = [(Ab - As) / Ab] \times 100$$

Ab: blank absorbance,
As: sample absorbance

### 2.7. Inhibitory activity of the tyrosinase

Tyrosinase assay was used to measure tyrosinase inhibition effect of the extract on both L-tyrosine and dihydroxy phenylalanine (DOPA). Briefly, normal human melanocyte cells were cultured in 24-well plates. After being treated with an individual herbal preparation for 24 h, cells were washed with 1% PBS and lysed with PBS (pH 6.8) containing 1% Triton X-100. Then, cells were disrupted by freezing and thawing, and lysates were clarified by centrifugation at 10,000×*g* for 10 min. Each well of a 96-well plate contained 40 μg protein, 2.5 mM-DOPA, and 0.1 M PBS (pH 6.8). After incubation at 37°C for 1 h, the absorbance was measured at 450 nm using an ELISA (enzyme-linked immunosorbent assay, Synergy HT, BIOTEK, USA) reader. Tyrosinase inhibition was calculated with the following

formula:

Tyrosinase inhibition (%) = [1 − (OD of sample / OD of control)] × 100

### 2.8. Inhibitory activity of elastase

An aliquot of 20 mL of Human leukocyte elastase solution containing sodium acetate buffer solution (50 mM, pH 5.3) and 25 ~400 μg/mL of sample were reacted in 48-well plates and 200 mL of p-nitroanilide (400 mM) was added. After reaction at 37°C for 20 min, an aliquot of 120 mL of reaction was added into 96 well plates. The absorbance was measured at 410 nm using an ELISA reader. Elastase inhibition was calculated with the following formula:

Elastase inhibition (%) = [1 − (OD of sample / OD of control)] × 100

### 2.9. Inhibitory activity of collagenase

An aliquot of 300 mL of collagen solution (0.25 mg/mL), 600 mL of sample, and 600 mL of collagenase solution (0.5 unit) were mixed with 1,500 mL of PBS (pH 6.0). After pre-incubation for 20 min in a dark room, the absorbance was measured at 280 nm and 200 nm with a fluorescence spectrophotometer (F-4500, Hitachi, Japan). Collagenase inhibition was calculated with the following formula:

Collagenase inhibition (%) = [1 − (OD of sample/OD of control)] × 100

## 3. RESULT AND DISCUSSION

To investigate the proximate composition of raw eggplant, moisture, ash, crude protein, crude lipid, crude fiber, anthocyanins and carbohydrate contents were measured. The proximate compositions are shown in Table 1. Moisture, ash, crude protein, crude lipid, crude fiber and carbohydrate contents were 94.05, 0.69, 0.62, 1.31, 2.85, and 0.48%, respectively. The pH is a determining factor in the ability of food to be preserved. Thus, a pH ranged between 3 and 6 is very favorable to the growth of yeasts and molds. pH of eggplant was 4.93. According to Ergezer and Gocke [18], the increases of pH could be ascribed to the reduction of available carboxylic groups of proteins, but also to the release of calcium and magnesium ions from proteins. Anthocyanins content was 0.26 mg/100g. The acidity indicates the maturity of the fruit (it decreases during maturation) and the ratio of sugars/acidity determines the gentle character, balanced or sour fruit. The acidity of eggplant was 1.32%. Fig. 1 is fatty acid composition of raw eggplant. The main fatty acids were 16:0, 18:0, and 18:2, which comprised approximately 85.4% of the total fatty acids. The unsaturated fatty acid can affect the physical properties of the membrane, such as fluidity and permeability [19]. Among various fatty acids, the 16:0 content was the highest, 39.2%, which was approximately 55.2% of polysaturated fatty acid. The total saturated fatty acid is 70.9%, which makes it strongly resistant to oxidative rancidity. The polyunsaturated fatty acid (PUFA)/ saturated fatty acid (SFA) ratio is generally used to evaluate the nutritional value of oil. The ratio

**Table 1.** Proximate composition of eggplant

| Proximate composition | Content |
|---|---|
| Moisture (%) | 94.05 |
| Ash (%) | 0.69 |
| Crude protein (%) | 0.62 |
| Crude lipid (%) | 1.31 |
| Crude fiber (%) | 2.85 |
| Carbohydrate (%) | 0.48 |
| Acidity (%) | 1.32 |
| Anthocyanins (mg/100g) | 0.26 |
| pH | 4.93 |

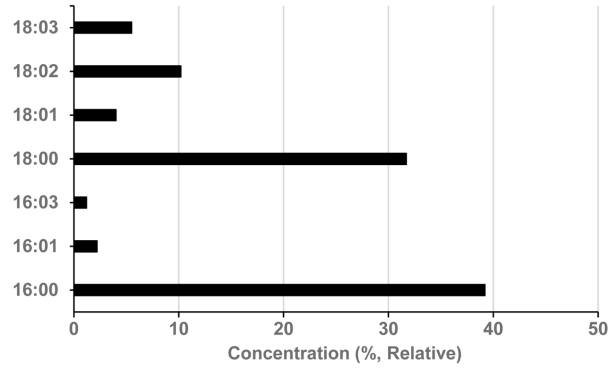


**Fig. 1.** Fatty acid composition of eggplant.

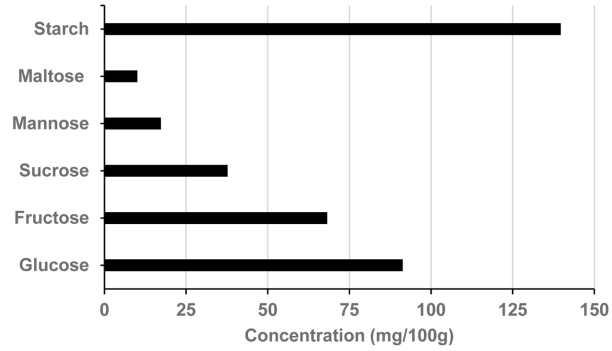


**Fig. 2.** Free sugar composition of eggplant.

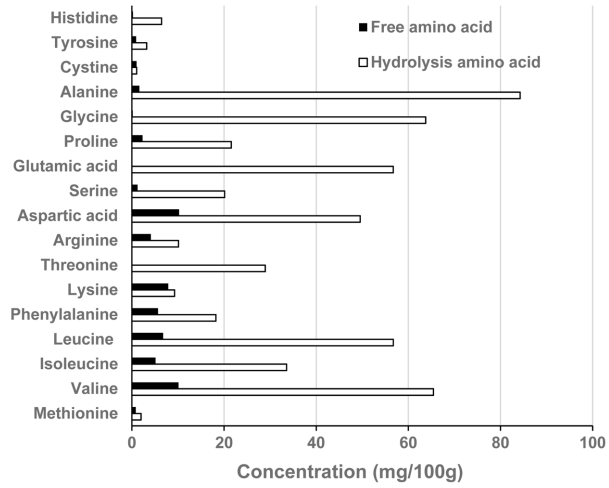


**Fig. 3.** Amino acid composition of eggplant.

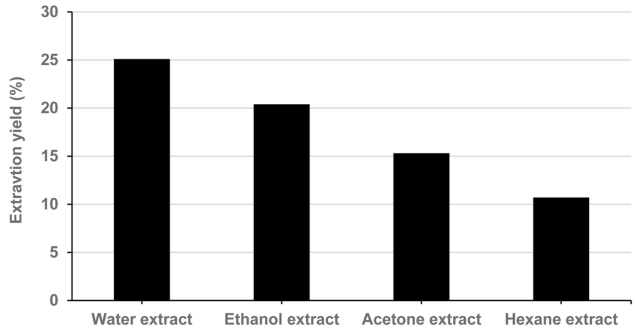


**Fig. 4.** Effect of various extractants on extraction yield.

of PUFA/SFA was 32.5%. These patterns of changes in eggplant fatty acids were similar to those obtained with those of tomato [20]. Fig. 2 is free sugar composition of raw eggplant. The main soluble sugars were glucose and fructose, representing an average 40.7% and 30.3% of the total soluble sugars content, respectively. Sucrose, mannose, and maltose were also identified, although their concentrations were much lower and accounted for 16.8, 7.7, and 4.5% of the total soluble sugars content, respectively. The content of starch was higher than the content in total soluble sugars. The ratio total soluble sugars/ starch was 0.62. The compositions of hydrolysis and free amino acids of raw eggplant were investigated and shown in Fig. 3. The most major components of the essential hydrolysis amino acid showed valine, leucine, and isoleucine, respectively. In the case of non-essential hydrolysis amino acids, they were alanine, glycine, glutamic acid, and aspartic acid, respectively. The ratios of essential hydrolysis amino acid and non-essential hydrolysis amino acid against total amino acid were 40.3 and 59.7%, respectively. Total content of hydrolysis amino acid was 531.0 mg/100g. Among different hydrolysis amino acids, the essential amino acid contents were order of valine, leucine> isoleucine> threonine> phenylalanine> lysine> methionine and their contents were 65.45, 56.73, 33.57, 28.94, 18.24, 9.28, and 1.98, mg/100g, respectively. The contents of non-essential amino acid were order of alanine> glycine > glutamic acid> aspartic acid> proline> serine> arginine> histidine > tyrosine> cystine and their concentrations were 84.25, 63.77, 56.72, 49.55, 21.58, 20.13, 10.11, 6.45, 3.21, and 1.04 mg/100g, respectively. On the other hand, in the case of free amino acid, valine, lysine and leucine were major components in essential amino acid and their contents were 10.11, 7.88, and 6.78 mg/100g. In the case of threonine, was not detectable. The ratio of essential free amino acid against total amino acid was 62.3%. Total content of hydrolysis amino acid was 58.39 mg/100g. Aspartic acid and arginine were major components in nonessential amino acid and their contents were 10.25 and 4.12 mg/100g. In the case of glutaminc acid, it was not detectable. The ratio of nonessential free amino acid against total amino acid was 37.7%. These results show that eggplant has the potential to be a resource for supplementation of essential amino acids. Phenolic compounds are commonly found in both edible and nonedible plants.

Various extractants such as water, ethanol, acetone, and hexane were used to investigate the extraction yield of thermal treated eggplant. The results are shown in Fig. 4. The extraction yield depended on the type of extractant. Higher extraction yields were obtained with an increase in solvent polarity. Especially, when water and ethanol were used, the extraction yields were 25.1 and 20.4%, respectively. However, when acetone and hexane were used, the extraction yields were 15.3 and 10.7%, respectively. These results indicate that the extraction yields were strongly affected by the solvent used for extraction. These results also indicate that the use of water to extract soluble components can be applied to the preparation of Korean herb medicine and the brewing of herbal tea. Therefore, as compared to other extractants, the information obtained using water extract would be more valuable for products used in human diets.

Crude extracts of fruits, herbs, vegetables, cereals, and other plant materials rich in phenolics are of increasing interest to the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food [21,22]. The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufacturers, and consumers in a trend that is moving towards functional foods with specific health effects [22]. Thus, it is important to consider the effects of the thermal treatment time of raw eggplant on the phenolic contents. The results are shown in Fig. 5. The total phenolic concentrations

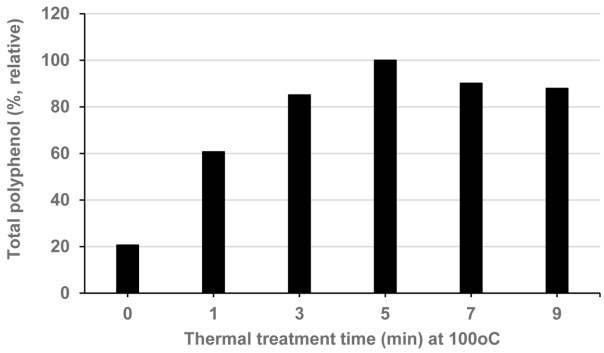


**Fig. 5.** Effect of thermal treatment time of eggplant on total phenol.

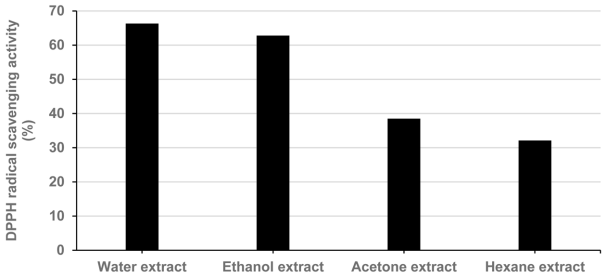


**Fig. 6.** Effect of various extracts of thermal treated eggplant on DPPH radical scavenging activity.

were dependent on the thermal treatment time. When thermal treatment time was increased from 1 to 5 min, the total phenol concentration was 60.7 to 100%. However, in the case of over 7 min of thermal treatment time, they did not increase. These results indicate that the total phenol content of eggplant were strongly affected by thermal treatment time.

Reactive oxygen species (ROS), which consist of free radicals such as superoxide anion and hydroxyl radicals and non-free radical species such as hydrogen peroxide and singled oxygen, are different forms of activated oxygen [23]. Therefore, living organisms possess a number of protective mechanisms against the oxidative stress and toxic effects of ROS. Antioxidants regulate various oxidative reactions naturally occurring in tissues and are evaluated as a potential anti-aging agent. Hence, antioxidants can terminate or retard the oxidation process by scavenging free radicals, chelating free catalytic metals and also by acting as electron donors [24]. Therefore, dietary intake of antioxidants is necessary and important. In order to investigate effect of various extracts obtained from thermal treated eggplant on DPPH radical scavenging activity, 9 mg/mL of water, ethanol, acetone, and hexane extract were used, respectively. The results are shown in Fig. 6. When water and ethanol extracts were used, the maximum DPPH radical scavenging activities were obtained,

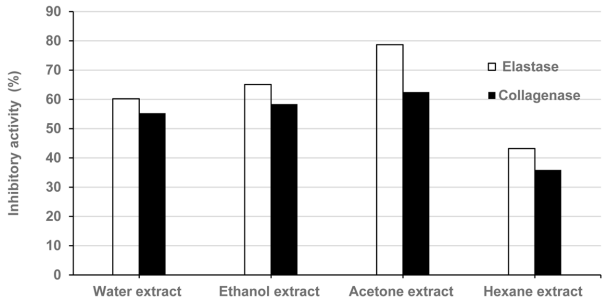


**Fig. 7.** Effect of various extracts of thermal treated eggplant on the inhibitory activity of elastase and collagenase.

66.3 and 62.8%, respectively. On the other hand, in the case of acetone and hexane extracts, they were 38.7 and 32.1%, respectively. These result suggest that water and ethanol extract of thermal treated eggplant have strong correlation between polyphenol and DPPH radical scavenging activity and have the potential as food additives to increase antioxidant activity in foods.

UV irradiation from sun produces free radicals and related reactive oxygen species (ROS) in human skin. These injure the DNA and extracellular matrix (ECM) in dermis of human skin. UV irradiation has been shown to stimulate fibroblast which secretes matrix metalloproteinases (MMPs) by cytokines. MMPs constitute more than 20 proteinase, and can degrade most components of ECM such as collagen, laminins, and elastins. Since collagen fibrils with elastin are responsible for the strength and resiliency of skin, their degradation causes wrinkles and skin aging [25]. To investigate the anti-wrinkle effect of thermal treated eggplant, water, ethanol, acetone, and hexane extracts on the inhibition effects of elastase and collagenase were studied. The inhibition effects of elastase and collagenase are shown in Fig. 7. Among various extracts, the acetone extract had the highest inhibition effect of elastase, followed by ethanol extract, water extract, and hexane extract. Especially, when acetone extract (15.0 mg/mL) was used, the maximum inhibition effect of elastase was 78.2%. For hexane extract, it was 13.2%. These results showed that the inhibition effect of elastase was strongly affected by the acetone extract concentration. In the case of inhibitory activity of collagenase of extracts, the acetone extract had the highest inhibition activity of collagenase, followed by ethanol extract, water extract, and hexane extract. Especially, when acetone extract was used, the maximum inhibition activity of collagenase was 62.5%. For haxane extract, it was 35.4%. These results showed that the effects of collagenase were affected by the acetone extract of thermal treated eggplant. Tyrosinase inhibitors are important constituents of cosmetics and skinlightening agents. Tyrosinase, a copper-containing monooxy genase, is a key enzyme that catalyzes melanin synthesis in melanocytes. It

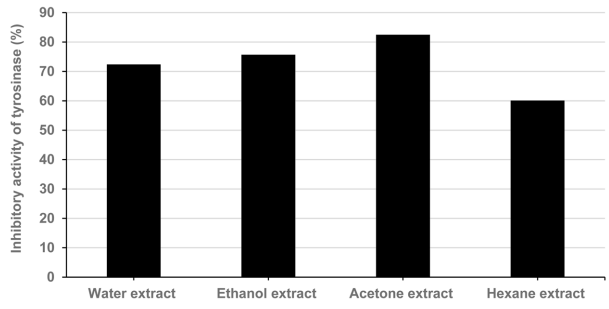


**Fig. 8.** Effect of various extracts of thermal treated eggplant on inhibitory activity of tyrosinase.

catalyzes two major reactions, including hydroxylation of tyrosine and oxidation of the o-diphenol product, l-dopa. Dopa oxidation produces a highly reactive intermediate that is further oxidized to form melanin by a free radical-coupling pathway [26]. To investigate the whitening effect of thermal treated eggplant, water, ethanol, acetone, and hexane extracts on the inhibitory activity of tyrosinase were studied. The inhibition effects of tyrosinase are shown in Fig. 8. Among various extracts, the acetone extract had the highest inhibition effect of tyrosinase, followed by ethanol extract, water extract, and hexane extract. Especially, when acetone extract (15.0 mg/mL) was used, the maximum inhibition effect of tyrosinase was 83.4%. In the case of hexane extract, it was 58.2%. In comparison, ascorbic acid, a naturally occurring cosmetic vehicle and whitening agent with tyrosinase inhibitory activity, has 94.2% at 2.0 mg/mL.

## 4. CONCLUSION

Eggplant (*Solanum melongena* L.) has been used worldwide as a vegetable side dish and medicinal stuff. The present study evaluated *in vitro* antioxidant and cosmetic activities of various solvents extracts of thermal treated eggplant. The phenolic content in eggplant were significantly affected by thermal treatment. The water and methanol extracts were the best one for DPPH radical scavenging activity. On the other hand, the acetone extract gave the highest inhibition activities of tyrosinase, elastase, and collagenase compared to other extracts. Therefore, *S. melongena* may be considered a source of important phytochemicals with important antioxidant and cosmetic properties.

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