RESEARCH ARTICLE

Anti-Aging Activities of Asparagus Gel Ethanol Extract in Cosmetic Gel Agent for Facial Skin

Henny Safrita Ginting,1 Edy Fachrial,2 I Nyoman Ehrich Lister,2 Adek Amansyah2
1Master Program of Biomedical Sciences, Universitas Prima Indonesia, Medan, Indonesia, 2Faculty of Medicine, Universitas Prima Indonesia, Medan, Indonesia

Abstract

Asparagus is a vegetable that contains phenolic compounds with antioxidant properties that scavenges aging-trigerring free radicals. This study aimed to investigate the components and anti-aging potentials of Ethanol Extract form Asparagus (EEA). The study was performed in February 2020 at the Pharmacy Laboratory, University of North Sumatera. The EEA was obtained through maceration using 96% ethanol. An antioxidant assay was performed and the total phenol and flavonoid content were determined using the spectroscopic method. Three gel formulas with different concentrations of EEA was prepared (F1: 1.5%, F2: 2.5%, and F3: 3.5%), and F0 was used as control. The parameters evaluated were moisture, oil content, texture, collagen, wrinkle, pigment, sensitivity, and pore. The result showed that asparagus had a moderate antioxidant activity (IC50: 118,992) with the total phenol and flavonoid contents of 15,9407 mg GAE/g and 3,2286 mg QE/g extract, respectively. The highest aging activities was seen in F3 (3.5%), followed by F2 (2.5%) and F1 (1.5%). The percentage of moisture, oil, texture, collagen, wrinkle, spot, sensitivity, and pore recovery were found to be 40.15%, 49.73%, 71.76%, 17.70%, 70.93%, 49.34%, 42.56% and 25.31%, respectively. Hence, it can be concluded that the EEA Gel at the highest concentration (3.5%) has a high content of phenol and flavonoid which can improve the skin moisture, oil content, texture, collagen, wrinkles, spots, sensitivity, and pores, which promotes anti-aging activities.

Key words: Anti-aging, antioxidant, asparagus

Pengaruh Ekstrak Etanol Asparagus sebagai Sediaan Kosmetik Gel Anti-Penuaan Terhadap Kulit Wajah

Abstrak

Asparagus adalah sayuran yang mengandung senyawa fenol dengan kemampuan sebagai antioksidan. Antioksidan dapat menangkal radikal bebas penyebab penuaan. Penelitian ini bertujuan untuk mengetahui kandungan antioksidan dan potensi efek anti-aging pada EEA. Penelitian dilakukan pada bulan Februari 2020 di Laboratorium Farmasi Universitas Sumatera Utara. Sementara itu, ekstrak diperoleh secara maserasi menggunkaan etanol 96%. Pengujian antioksidan dan penentuan kadar total fenol dan flavonoid dilakukan dengan metode spektroskopi. Tiga formula gel dengan konsentrasi EEA yang berbeda disiapkan (F1: 1,5%, F2: 2,5%, dan F3: 3,5%), dan F0 digunakan sebagai kontrol. Parameter uji berupa kadar air, kadar minyak, tekstur, kolagen, keriput, pigment, sensitivitas, dan pori. Hasil penelitian menunjukkan bahwa asparagus memiliki aktivitas antioksidan dan sedang (IC50: 118,992) dengan kandungan total fenol dan flavonoid masing-masing sebesar 15,9407 mg GAE/g dan 3,2286 mg QE/g ekstrak. Aktivitas penuaan tertinggi terlihat pada F3 (3,5%), diikuti oleh F2 (2,5%) dan F1 (1,5%). Persentase kelembaban, minyak, tekstur, kolagen, kerutan, flek, sensitivitas, dan penulihan pori ditemukan tiap-tiap 40,15%, 49,73%, 71,76%, 17,70%, 70,93%, 49,34%, 42,56% dan 25,31%. Simpulan, gel EEA pada konsentrasi tertinggi (3,5%) memiliki kandungan fenol dan flavonoid yang tinggi yang dapat memperbaiki kelembaban kulit, kandungan minyak, tekstur, kolagen, kerutan, flek, sensitivitas, dan pori-pori, yang menunjukkan aktivitas anti-penuaan.

Kata kunci: Anti penuaan, antioksidan, asparagus

Corresponding Author: Henny Safrita Ginting, Master Program of Biomedical Sciences, Universitas Prima Indonesia, Medan, Indonesia, Email: yysunpri@gmail.com
Introduction

Aging is a natural process that cannot be avoided by humans due to anatomical and physiological damages starting from blood vessels and other organs to the skin. The extrinsic aging (photoaging) of the skin is mainly affected by ultraviolet (UV) rays, and the exposure to UV radiation from sunlight is the biggest factor contributing to 90% of premature aging symptoms. The thinning of skin layers due to sun exposure and clumping of pigments (melanocyte cells) causes spots and dry skin. Photoaging causes 80% of skin aging problems by activating cytokines and metalloprotein collagenases and stimulating free radicals. Collagen and elastin (ELN) form cross-link in the skin, causing loss of elasticity, thinning the epidermal layer and wrinkles. Furthermore, collagen is the largest part of the dermis, which contributes about 70% of the skin dry mass; hence its damage is a major cause of wrinkling, loss of elasticity, and sagging.

The two main regulators of collagen formation by fibroblast cells are transforming growth factor (TGF-β) and activator protein (AP-1). TGF-β is a cytokine that stimulates collagen production, while AP-1 is a transcription factor that inhibits collagen production and stimulates collagen breakdown. Intrinsic aging plays a role in decreasing TGF-β and accumulation of Reactive oxygen species (ROS), while extrinsic aging, which is mainly caused by UV radiation (photoaging), causes an increase in ROS production in the dermis layer. Furthermore, ROS triggers a series of chain molecular reactions, thereby increasing the formation of AP-1, which stimulates the transcription process of Matrix metallopeptidase (MMP) enzyme in collagen degradation and inhibits collagen synthesis by inhibiting the type 2 receptors of TGF-β. Antioxidants can prevent aging by acting as an antidote to free radicals from photoaging by working synergistically to protect cells and organ systems from damage. Asparagus contains phenolic compounds with antioxidant properties that cleanse toxic and acne trigger substances from photoaging on the face. Natural ingredients have identified benefit for the dermatologic disorder, and it has been used traditionally over the last 20 years. This active natural ingredient can be formulated into cosmetics that can be used safely and have lower side effects than synthetics cosmetics. This category of cosmetic preparation is a gel, which is non-sticky, easy to wash, leaves no oil on the skin, and has stable viscosity during storage. Skincare for aging problems is best carried out at the earliest opportunity for a healthy and well-maintained facial skin. Total phenolic and total flavonoids are positively correlated with antioxidant activity. Therefore, this study aimed to investigate the antioxidant activity, total phenolic, and flavonoid content from the ethanol extract of Asparagus (EEA) and its anti-aging potential.

Methods

This study was performed in February 2020 at the Pharmacy Laboratory, University of North Sumatera. This study evaluated antioxidant activity from the EEA by DPPH (2,2-diphenyl-1-picrylhydrazyl) as well as total phenolic and flavonoid content Folin-Ciocalteau and Aluminum chloride.

Furthermore, EEA was formulated into a gel (F0=Gel Base, F1=Gel of 1.5% EEA, F2=Gel of 2.5% EEA, and F3=Gel of 3.5% EEA), and efficacy of gel was evaluated by double-blinding clinical trial against 12 volunteers who have been informed about the purpose and procedure of this study. These volunteers, as the sample was limited by inclusion and exclusion criteria. Inclusion criteria were healthy women or men, productive age (20–25 years), no history of allergy-related illness, and willing to receive treatment using gel for 4 weeks, twice daily (day and night). Exclusion criteria were irritation of the gel, history of an allergy-related illness, and in the care of another dermatologist. The evaluated parameters included moisture, oil content, texture, collagen, wrinkles, pigments, sensitivity, and pores, which were evaluated every week for a month. This clinical trial procedure has been approved by the Health Research Ethics Commission, Universitas Prima Indonesia with Letter No. 022/KEPK/UNPRI/I/ 2020.

Preparation of EEA was begun by washing and drying the asparagus at room temperature. The dried Simplicia was then blended into Simplicia powder and extracted. The extraction was performed by maceration using 96% ethanol for 7 days at room temperature. The filtrate from the maceration evaporated by a rotary evaporator and obtained a concentrated form of EEA.

The obtained EEA undergo a phytochemical screening, according to the Indonesian Herbal Pharmacopeia. These phytochemical was screened by some reagent, such as dragendorf, Mayer, bouchardat for alkaloids, AlCl₃ for tannins, Lieberman

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Ethanol Extract of Asparagus (EEA) contains some phytochemicals as a secondary metabolite, including glycoside, steroid/triterpenoids, flavonoid, tannin, and saponin. On the other hand, the result of the antioxidants assay against the EEA as sample and vitamin C as positive control by DPPH methods was expressed as IC$_{50}$ of DPPH. The IC$_{50}$ of EEA was 118.992 μg/mL, while vitamin C as a positive control had an IC$_{50}$ value of 2.693 μg/mL. This indicated that the component in EEA had a moderate antioxidant activity (100–150 μg/mL), while vitamin C had a very strong antioxidant activity (less than 50 μg/mL).

### Results

Ethanol Extract of Asparagus (EEA) contains some phytochemicals as a secondary metabolite, including glycoside, steroid/triterpenoids, flavonoid, tannin, and saponin. On the other hand, the result of the antioxidants assay against the EEA as sample and vitamin C as positive control by DPPH methods was expressed as IC$_{50}$ (μg/mL) and shown in Table 2.

The IC$_{50}$ of EEA was 118.992 μg/mL, while vitamin C as a positive control had an IC$_{50}$ value of 2.693 μg/mL. This indicated that the component in EEA had a moderate antioxidant activity (100–150 μg/mL), while vitamin C had a very strong antioxidant activity (less than 50 μg/mL).

### Table 1 The Formulation of gel EEA

| Material               | Concentration (%) |
|------------------------|-------------------|
| HPMC                   | 1%                |
| Propylene glycol       | 10%               |
| Glycerin               | 5%                |
| Nipagin                | 0.1%              |
| Triethanolamine        | Quantum sufficit  |
| Distilled Water        | 100%              |
Moreover, EEA underwent to determine total phenolic content and Total Flavonoid Content. Total phenolics and flavonoid content were expressed as Gallic Acid Equivalent (GAE) and Quercetin Equivalent (QE) for each gram of extract. Total phenolic and flavonoid content from EEA was shown in Table 3.

After that, EEA was formulated into a gel for the clinical trial. The irritation test showed no sign of a reaction, such as redness, itching, and skin roughness among volunteers. Therefore, EEA gel preparations are safe to be used. Hence, all volunteers could apply the gel for 4 weeks, and the analysis of the parameter was shown in Table 4, and the percentage of recovery after using AAE gel was shown in Figure.

Based on Figure, percentage of recovery from all parameters was shown a similar pattern. FIII formulation shown the highest percentage of recovery for moisture (40.1%), oil content (49.7%), texture (71.7%), collagen (17.7%), wrinkles (70.9%), spots (49.3%), sensitivity (42.6%), and pore (25.3%) than other formulation, as the opposite the lowest percentage of recovery was revealed by F0.

### Discussion

The result of this study indicates various pharmacology properties, not only increase skin

| Table 2 The Result of Antioxidant Assay |
|-----------------------------------------|
| Group       | Regression Equation          | IC$_{50}$ (µg/mL) |
| EEA         | $y=0.3507x + 8.260$           | 118.992           |
| Vitamin C   | $y=17.548x + 2.7315$          | 2.6935            |

| Table 3 Results of the Total Phenolic and Total Flavonoid Content of EEA |
|-------------------------------------------------|
| Phytochemical       | Concentration (ppm) | Volume (L) | Dilution Factor | Mass of Sample (g) | Content |
|---------------------|---------------------|-------------|-----------------|--------------------|---------|
| Total Phenolics     | 16,068              | 0.025       | 1               | 0.025              | 15,940 GAE/g extract |
| Total Flavonoid     | 3,2545              | 0.025       | 1               | 0.025              | 3,2286 QE/g extract  |

Table 4 Results for the Effectiveness of EEA Gel

| Parameter         | Testing Time | F0               | F1               | F2               | F3               | P     |
|-------------------|--------------|------------------|------------------|------------------|------------------|-------|
| Moisture          | Before       | 74.67±2.08       | 76.67±2.08       | 63.33±4.16       | 43.00±9.85       | 0.021 |
| After             | 89.67±1.52   | 98.00±0.00       | 92.00±3.46       | 72.33±18.71      | 0.021            |
| Oil Content       | Before       | 59.67±2.88       | 62.00±3.46       | 63.00±2.64       | 73.00±8.72       | 0.148 |
| After             | 46.33±3.51   | 43.00±3.61       | 39.67±1.52       | 36.66±4.04       | 0.044            |
| Texture           | Before       | 9.33±1.53        | 8.67±1.53        | 10.00±0.00       | 8.33±0.57        | 0.255 |
| After             | 4.33±0.57    | 3.67±0.57        | 4.00±1.00        | 2.33±0.57        | 0.083            |
| Collagen          | Before       | 86.67±2.31       | 83.67±1.53       | 81.33±1.15       | 79.00±0.00       | 0.022 |
| After             | 94.67±0.21   | 97.33±1.15       | 97.67±0.57       | 96.00±1.00       | 0.087            |
| Wrinkles          | Before       | 8.00±1.00        | 8.00±1.00        | 8.67±1.52        | 9.00±1.00        | 0.597 |
| After             | 5.00±1.00    | 3.67±0.57        | 3.67±0.57        | 2.67±1.15        | 0.111            |
| Spots             | Before       | 26.33±0.57       | 24.33±1.52       | 27.00±0.00       | 31.33±4.16       | 0.020 |
| After             | 21.33±0.57   | 16.33±1.52       | 15.00±0.00       | 16.00±3.60       | 0.059            |
| Sensitivity       | Before       | 20.67±1.15       | 22.67±2.51       | 23.33±2.08       | 24.33±0.57       | 0.188 |
| After             | 15.67±1.15   | 15.33±1.52       | 14.33±1.15       | 14.00±1.73       | 0.483            |
| Pore              | Before       | 1.00±0.00        | 1.00±0.00        | 1.00±0.00        | 72.67±16.56      | 0.013 |
| After             | 1.00±0.00    | 1.00±0.00        | 1.00±0.00        | 54±10.53         | 0.013            |

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moisture and collagen but also reduce oil content, texture, wrinkles, spot, sensitivity, and pore. This pharmacology properties were due to Ethanol Extract of Asparagus (EEA) having moderate antioxidant activity by scavenging DPPH. The antioxidant activity of EEA was supported by the total phenolics and flavonoid content, which were 15,9407 GAE/g extracts and 3,2286 QE/g extracts, respectively. The in vitro study was to show potential anti-aging properties from EEA. Moreover, the EEA was evaluated as gel preparation in 3 different formulations (F1, F2, and F3), and these gels of EEA were also showed an improvement in the facial skin parameter after 4 weeks (p-value <0.05).

The gel penetration through the skin occurred by percutaneous absorption that entered the bloodstream. Meanwhile, drug penetration through the skin occurred via the transdermal route (stratum corneum) and the transfollicular route (sweat and sebum gland pores). Propylene glycol is an enhancer that interacts with stratum corneum lipids and water to increase hydration in skin tissue, thereby increasing the delivery of hydrophilic and lipophilic drugs, influencing drug solubility in the stratum corneum and affecting the carrier partition into the membrane. In addition, increased penetration in gel preparations accelerates the effectiveness of medicinal ingredients15. The antioxidant compounds in asparagus were flavonoids and tannins, with a moderate antioxidant activity as indicated by the IC50 value of ethanol extract by 118.992 µg/mL, which was supported by the phenol and flavonoid content. Phenolic compounds are a source of natural antioxidants. Phenol and flavonoid compounds have a linear contribution to antioxidant activity; therefore, the higher the levels, the better the antioxidants.16 Before applying the gel, the patient's skin condition was dry epidermis-dermis, oily, perfect texture, sufficient collagen fiber, no wrinkles, spots, sensitive, and no serious pores. After 4 weeks of gel application, there was an improvement in skin condition, which became moist, oily balance, perfect texture, sufficient collagen fiber, no wrinkles, normal spots, normal facial skin sensitivity, and a decrease in pore size. Asparagus keeps facial skin moist by maintaining sebum production in the stratum corneum and removes fat in oily skin.2,17 The use of 3.5% asparagus ethanol extract gel (FIII) provided the best effect in all parameters (Figure 2)

Antioxidants and flavonoids work to stimulate the formation and production of skin collagen, prevent collagen degradation. In addition, it maintains and improves facial skin texture by preventing the increase in ROS in the dermis layer, thereby inhibiting the formation of AP-1 and the MMP enzyme. Increased collagen maintains...
skin elasticity, flexibility, and smoothness.\textsuperscript{10} The development of asparagus in a gel preparation based on Hydroxypropyl methylcellulose, which is a cellulose derivative increases the stimulation of growth factors, such as epidermal growth factor (EGF), Fibroblast growth factor (FGF), and Platelet-derived growth factor (PDGF). Growth factors play an important role in regulating normal growth and development by stimulating cell division, maintaining the tissue repair phase, accelerating skin regeneration, and stimulating collagen formation.\textsuperscript{16,19} Furthermore, flavonoids from EEA inhibit the pigmentation process or the appearance of spots by directly inhibiting tyrosinase activity in the melanogenesis process. Antioxidants can keep facial skin from overreaction that interferes with its health and prevents irritation and allergies. In addition, enlarged pores may be reduced by regular exfoliation and collagen formation, which improves skin.\textsuperscript{20} Hence, it can be concluded that Asparagus not only have moderate antioxidant activity by scavenging DPPH due to the presence of phenolic and flavonoid. Moreover, the highest concentration of EEA Gel (3.5\%) shown the highest percentage of skin moisture, oil content, texture, collagen, wrinkles, spots, sensitivity, and pore, which promotes an anti-aging activity.

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