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"Development of Koenigia alpina Extract as an Anti-Aging Agent and Its Potential Applications in Cosmetics"

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1. Introduction

Skin aging is classified into intrinsic aging (a decline in internal physiological functions) and extrinsic aging (caused by external factors like UV radiation and pollution)[1]. Repeated exposure to UV radiation causes photoaging, leading to skin dryness, reduced elasticity, hyperpigmentation, wrinkles, and sagging[2, 3]. UV radiation generates reactive oxygen species (ROS), which can damage skin cells and accelerate aging[4]. Melanin is produced to protect the skin from UV radiation, but excessive melanin production can cause hyperpigmentation disorders[5]. Plant-derived natural compounds are increasingly in demand for cosmetics due to consumer awareness of natural ingredients[6]. Previous studies have shown that extracts and components from plants in the Polygonaceae family have potential as antiaging and whitening ingredients in cosmetics[7, 8].

This study investigates the potential of *Koenigia alpine* (*K. alpina*), a plant in the Polygonaceae family, as a cosmetic ingredient, by evaluating its antioxidant, anti-inflammatory, whitening, and antiaging effects. In addition, the principal constituents of *K. alpina* were identified, and the antioxidant, anti-inflammatory, and wound-healing properties of the active compounds were evaluated to assess the potential applicability of *K. alpina* and its major bioactive components as functional cosmetic ingredients.

2. Materials and Methods

The dried *K. alpina* was ultrasonically extracted with 70% ethanol at room temperature for 3 hr, followed by concentration to obtain the 70% EtOH extract. The concentrated extract was further fractionated three times with ethyl acetate (EA) and water (1:1) to yield the EA fraction. Part of the EA fraction (containing flavonoid glycosides) underwent acid hydrolysis to remove sugars, resulting in the aglycone fraction. The extract and fractions were concentrated under reduced pressure, freeze-dried, and used in experiments in powder form. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radicals and H_2O_2 were used to evaluate the free-radical scavenging activity of *K. alpina* extract and its protective effect against reactive oxygen species (ROS)-induced cell damage. The whitening and anti-aging effects of the extracts were evaluated via tyrosinase inhibition, and collagenase and elastase inhibition, respectively. The anti-inflammatory effect was confirmed by evaluating nitric oxide (NO) production in RAW 264.7 cells. The main components of *K. alpina* were analyzed by high-performance liquid chromatography (HPLC) according to the analytical conditions shown in Table 1. In addition, the antioxidant, anti-inflammatory, and wound healing effects of the identified active components were further

evaluated using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging assay, nitric oxide (NO) production in RAW 264.7 cells, and scratch assay in HDF cells, respectively.

Table 1. HPLC conditions for separation of the ethyl acetate fraction from the *K.alpina* extract.

Column	Agilent TC-C18 (4.6 × 250 mm, 5 µm)		
Detector	365 nm		
Flow rate	1.0 mL/min		
Injection volume	20µL		
Column temperature	30◦C		
Mobile phase conditions for HPLC gradient elution	Time (min)	2% AA in water (%)	0.5% AA in 50% ACN (%)
	0	80	20
	35	40	60
	50	40	60
	55	80	20
	60	80	20

Abbreviations: AA, acetic acid; ACN, acetonitrile.

3. Results

3.1. Free Radical Scavenging Activity of K.alpina Extract

The free-radical scavenging activities (FSC_{50}) of the 70% EtOH extract was found to be 134.2 µg/mL, while the EA and aglycone fractions exhibited stronger antioxidant activities at 47.5 µg/mL and 47.1 µg/mL, respectively (Figure 1).

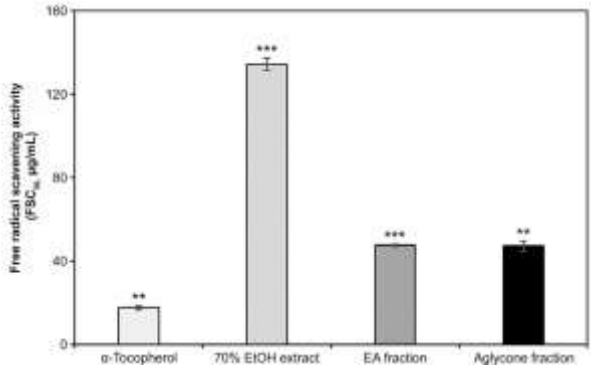


Figure 1. Free-radical scavenging activities of extract and fractions of *K. alpina*. Each value represents the mean ± SD of three independent experiments. Different symbols indicate significant differences (**p < 0.01, ***p < 0.001) based on ANOVA with Tukey's HSD test.

3.2. Tyrosinase Inhibition Activity of K.alpina Extract

Tyrosinase inhibition activity (IC_{50}) was 0.98 mg/mL for the 70% EtOH extract, 0.38 mg/mL for the EA fraction, and 0.72 mg/mL for the aglycone fraction (Figure 2).

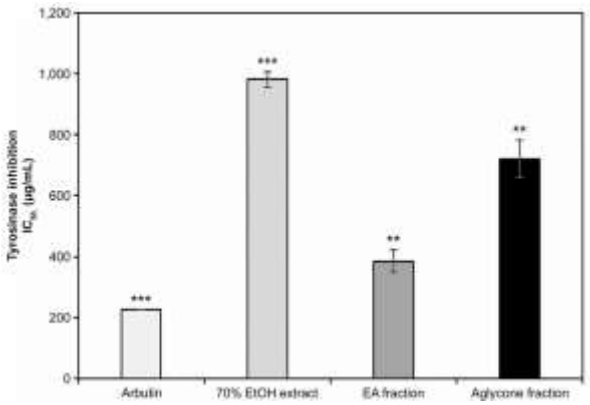


Figure 2. Tyrosinase inhibitory activities of extract and fractions from *K. alpina*. Each value represents the mean ± SD of three independent experiments. Different symbols indicate significant differences (**p < 0.01, ***p < 0.001) based on ANOVA with Tukey's HSD test.

3.3. Effects of K.alpina Extract on ROS-Induced Damage in HaCaT Cells

Cells were treated with 10 mM H_2O_2 for 2 hr and stained with MTT to compare the number of survival cells between treated and nontreated groups. In hydrogen peroxide (H_2O_2)-induced oxidative stress in HaCaT cells, the 70% EtOH extract demonstrated 87% cell protection, and both the EA and aglycone fractions showed 90% protection, compared to the 78% viability of the H_2O_2-treated group (Figure 3).

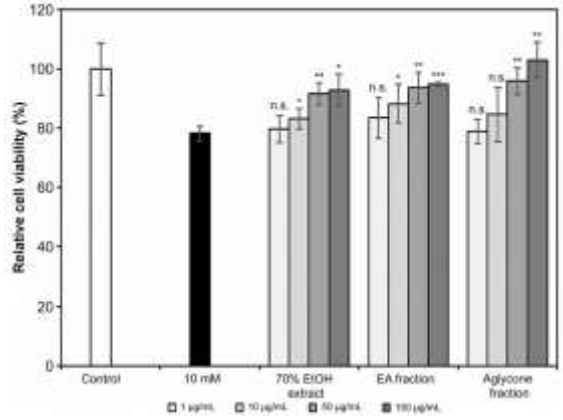
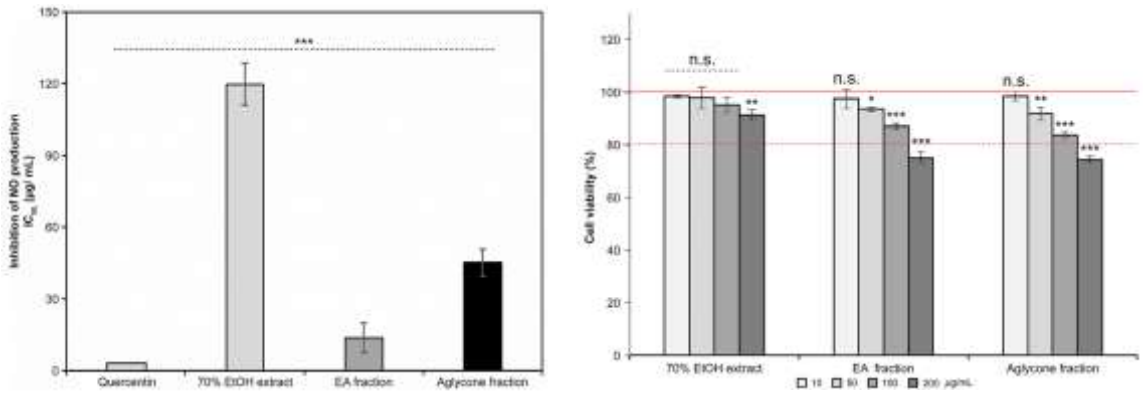


Figure 3. Cellular protective effects of extract and fractions from *K.alpina* on H_2O_2-induced cell damage in HaCaT cells. Each value represents the mean ± SD of three independent experiments. Different symbols indicate significant differences (*p < 0.05, **p < 0.01, ***p < 0.001) based on ANOVA with Tukey's HSD test.

3.4. Anti-Inflammatory Activity of K.alpina Extract on Macrophages

Inhibition of nitric oxide (NO) production in LPS-induced RAW 264.7 cells was concentration-dependent, with IC_{50} values of 119.8 μg/mL for the 70% EtOH extract, 13.6 μg/mL for the EA fraction, and 45.1 μg/mL for the aglycone fraction. The EA fraction exhibited the most potent anti-inflammatory activity. The anti-inflammatory activity was evaluated at concentrations that did not exhibit cell cytotoxicity. (Figure 4).



(**a**) (**b**)

Figure 4. Anti-Inflammatory Action of *K.alpina* Extract and frations on Macrophages. (a) Effects of extract and fractions from *K. alpina* on lipopolysaccharide (LPS)-induced NO production in RAW 264.7 cells. (b) Effects of *K. alpina* extract and fractions on the viability of RAW 264.7 cells. Each value represents the mean ± SD (n = 3). Different symbols indicate statistically significant differences (*p < 0.05, **p < 0.01, ***p < 0.001), and n.s. indicates no significant difference, based on one-way ANOVA followed by Tukey's HSD test.

3.5. Collagenase and Elastase Inhibition Activity of K. alpina Extract

Collagenase inhibition (IC_{50}) was 0.39 mg/mL for the 70% EtOH extract, 0.21 mg/mL for the EA fraction, and 0.22 mg/mL for the aglycone fraction (Figure 5). Elastase inhibition (IC_{50}) was 7.58 mg/mL for the 70% EtOH extract, 1.03 mg/mL for the EA fraction, and 0.57 mg/mL for the aglycone fraction, with the EA fraction showing the greatest inhibition in all assays (Figure 6).

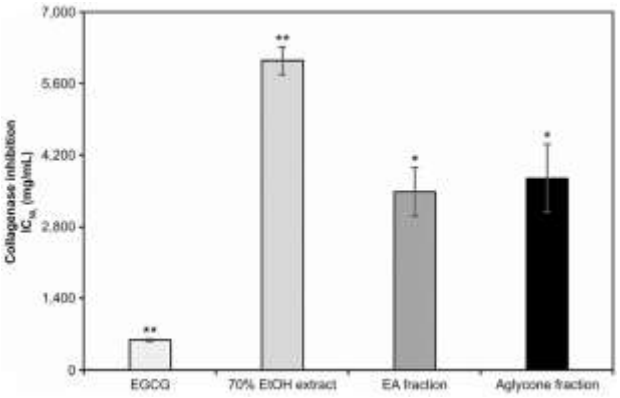


Figure 5. Collagenase inhibitory activities of extract and fractions from *K. alpina*. Each value represents the mean ± SD of three independent experiments. Different symbols indicate significant differences (*p < 0.05, **p < 0.01) based on ANOVA with Tukey's HSD test.

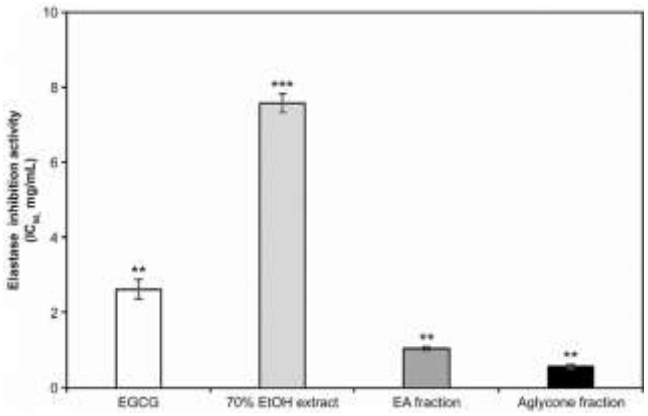


Figure 6. Elastase inhibitory activities of extract and fractions from *K. alpina*. Each value represents the mean ± SD of three independent experiments. Different symbols indicate significant differences (**p < 0.01, ***p < 0.001) based on ANOVA with Tukey's HSD test.

3.6. *Component Analysis*

The HPLC chromatogram of the EA fraction of the *K. alpina* extract is shown in Figure 7. The retention time (RT) of the Peak 1 component was 31.62 min; the RT of the Peak 2 component was 36.05 min; the RT of the Peak 3 component was 46.90 min. Peak 1, Peak 2, and Peak 3 were identified as quercitrin, quercetin, and cardamonin, respectively. These three compounds were determined to be the main components of the *K. alpina* extract.

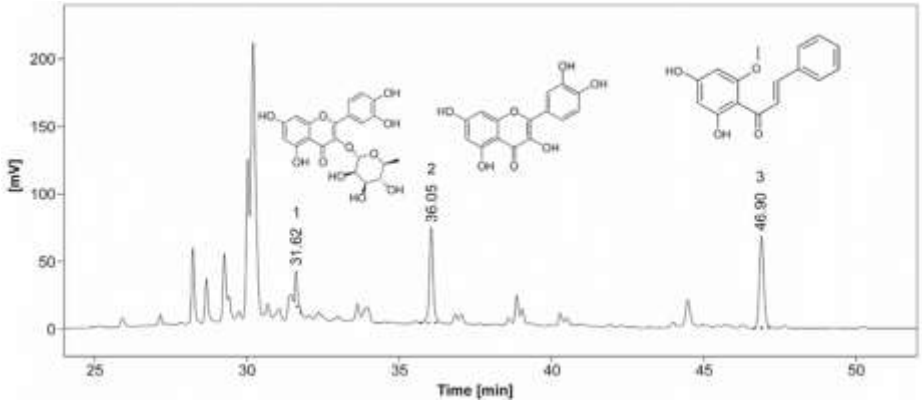


Figure 7. HPLC chromatograms of the ethyl acetate fraction from *K.alpina* extract at a λmax of 365 nm. (1) Quercitrin, (2) Quercetin, (3) Cardamonin.

3.7. Free Radical Scavenging Activity of K. alpina Extract and Cardamonin

The ABTS-radical scavenging activities of the *K. alpina* extract and cardamonin were evaluated based on their FSC_{50} values, which were 8.89 μg/mL and 1.50 μg/mL, respectively. Cardamonin exhibited a stronger antioxidant activity compared to the extract (Figure 8).

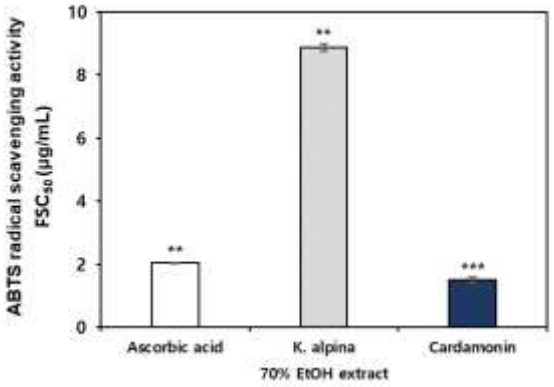
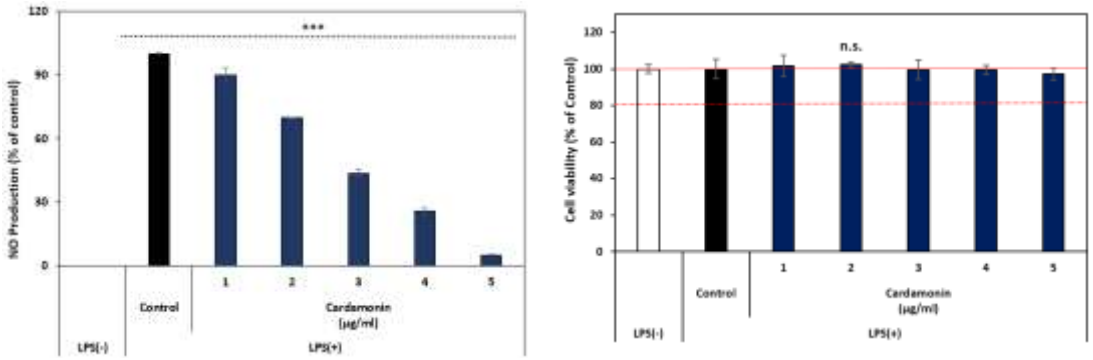


Figure 8. ABTS-radical scavenging activities of extract of *K. alpina* and Cardamonin. Each value represents the mean ± SD of three independent experiments. Different symbols indicate significant differences (**p < 0.01, ***p < 0.001) based on ANOVA with Tukey's HSD test.

3.8. Anti-Inflammatory Activity of Cardamonin on Macrophages

Cardamonin inhibited nitric oxide (NO) production in LPS-stimulated RAW 264.7 cells in a dose-dependent manner, with an IC_{50} value of 2.85 μg/mL. The anti-inflammatory activity was evaluated at concentrations that did not exhibit cell cytotoxicity (Figure 9).

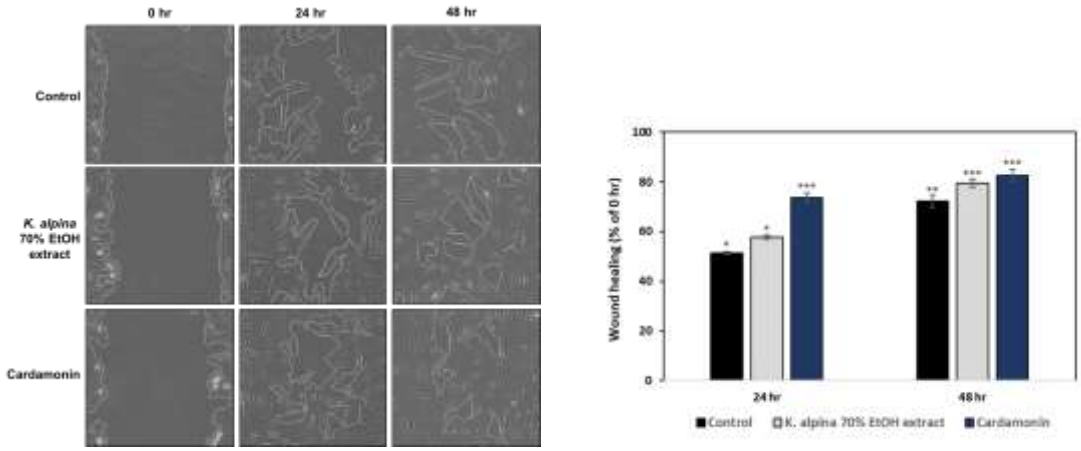


(**a**) (**b**)

Figure 9. Anti-Inflammatory activities of Cardamonin on Macrophages. (a) Effects of Cardamonin on lipopolysaccharide (LPS)-induced NO production in RAW 264.7 cells. (b) Effects of *K. alpina* extract and Cardamonin on the viability of RAW 264.7 cells. Each value represents the mean ± SD (n = 3). Different symbols indicate statistically significant differences (*p < 0.05, **p < 0.01, ***p < 0.001), and n.s. indicates no significant difference, based on one-way ANOVA followed by Tukey's HSD test.

3.9. *In Vitro Scratch Wound Healing Assay of K. alpina Extract and Cardamonin*

The wound healing effect of the 70% EtOH extract of *K. alpina* (100 μg/mL) was 58% at 24 hr and 79% at 48 hr, relative to the initial wound area. Cardamonin (0.5 μg/mL) also showed enhanced wound healing, with rates of 74% at 24 hr and 83% at 48 hr. In contrast, the control group exhibited wound healing rates of 51% and 72%, respectively, suggesting that both *K. alpina* extract and cardamonin were more effective than the control in promoting wound healing (Figure 10).



(**a**) (**b**)

Figure 10. Wound healing effects of *K. alpina* extract and cardamonin in HDF cells as evaluated by scratch assay. (a) Representative images of wound healing assay at each time points. (b) Percentage of wound healing in HDF cells calculated based on the blank area at 0 hr using Image J software. Each value represents the mean ± SD of three independent experiments. Different symbols indicate significant differences (*p < 0.05, **p < 0.01, ***p < 0.001) based on ANOVA with Tukey's HSD test.

4. Discussion

In this study, we demonstrated that the EtOH extract and its fractions from *K. alpina* exhibit significant DPPH radical scavenging, collagenase/elastase/tyrosinase inhibition, antiinflammatory, and cellular protective effects. The *K. alpina* extract and fractions showed robust antioxidant activity comparable to that of α-tocopherol, a potent antioxidant. Additionally, they exhibited concentration-dependent cellular protective effects against ROS-induced oxidative damage in HaCaT cells. The *K. alpina* extract and fractions inhibited tyrosinase in a concentration-dependent manner, with the EA fraction showing inhibition activity as effective as *Glycyrrhiza glabra* extract, a well-known whitening material.

Furthermore, the extract and fractions increased collagenase and elastase inhibitory activity. Collagen and elastin, vital dermal components, maintain skin flexibility and elasticity. These ECM proteins are gradually reduced by degrading enzymes (collagenase and elastase) with age, leading to skin wrinkles [9, 10]. The *K. alpina* fractions showed superior elastase inhibition activity compared with the positive control EGCG. Cell senescence, driven by inflammatory mechanisms, can be delayed by natural polyphenols [11, 12]. In the present study, both *K. alpina* fractions showed significant inhibition of NO production, with the EA fraction displaying the greatest inhibitory activity, comparable to that of quercetin. These results indicate that the *K. alpina* extract and fractions can inhibit active oxygen, skin aging enzyme activity, and inflammageing.

The significant activities of the *K. alpina* extract observed in this study can be attributed to its antioxidant, antiaging, whitening, cell protection, and anti-inflammatory effects. External stimuli, including UV radiation, accelerate the skin's natural aging process, characterized by wrinkles and atypical pigmentation [13]. Our findings provide evidence of *K. alpina* extract's efficacy as a possible skin treatment and will contribute to further investigation and development of this candidate substance for application as a natural cosmetic material effective against skin aging and diseases.

Active components such as isoquercitrin, quercitrin, quercetin, and cardamonin were identified in *K. alpina* extract using HPLC analyze. Especially, cardamonin, one of the main components, was found to exhibit significant antioxidant, anti-inflammatory and wound healing properties, contributing to the overall bioactivity of *K. alpina* extracts. These results highlight the promising role of cardamonin as an active ingredient in functional anti-aging and anti-inflammatory cosmetic formulations.

5. Conclusion

K. alpina extracts and their fractions demonstrated antioxidant, anti-aging, whitening, and anti-inflammatory activities, indicating their potential as functional cosmeceutical agents. Among the active compounds, cardamonin exhibited potent antioxidant, anti-inflammatory, and wound healing effects, supporting its key role in the overall bioactivity of *K. alpina*.

These biological effects can be attributed to the antioxidant, anti-aging, whitening, cytoprotective, and anti-inflammatory properties of the extracts. In particular, the 70% EtOH extract, EA fraction, and aglycone fraction show promise for use as natural cosmetic ingredients targeting skin aging, pigmentation, and ROS-induced inflammatory conditions.

6. Reference

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