PHYSIOLOGICAL ACTIVITIES OF ERUCA SATIVA EXTRACT

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Abstract— Eruca sativa extract showed a low cytotoxicity against murine melanoma B16F10 cells. In little or no cytotoxicity at concentrations, Eruca sativa extract showed the inhibition of tyrosinase activity (ID50, 132.54 mg/l) and decreased melanin content (ID50, 158.90 mg/l). In addition, the treatment of Eruca sativa extract suppressed the protein expression of tyrosinase according to concentration.

Keywords— Eruca Sativa, Melanin, Tyrosinase Commas.

I. INTRODUCTION

Eruca sativa is called rocket plant and a member of the Brassicaceae, which is considered to be an important chemo-preventive plant family [1]. Several studies have shown that *Eruca sativa* has positive biological effects such as antioxidant and renal protective activity [2]. However, compared to many pharmacological studies, the effect of *Eruca sativa* extract on whitening function as skin therapeutic agent has not been reported. In this study, we investigated the applicability of functional materials by examining a variety of physiological activities with the extract of *Eruca sativa*.

II. EXPERIMENTAL METHODS

A. Tyrosinase activity assay

The tyrosinase activity assay was performed with mushroom tyrosinase because of its ready availability. Each sample was dissolved in DMSO and used for the experiment at 100 times dilution. 0.1 M potassium phosphate buffer (pH 6.8), 3 mM L-tyrosine solution with or without a sample chemical and 2,000 units/ml tyrosinase in aqueous solution were mixed. The mixture incubated at 37°C for 10 min and the reaction was monitored at 475 nm [3]. S standard reaction was conducted without only sample solution and C control reaction was conducted without only L-tyrosine solution. The percentage of activity of tyrosinase was calculated as follows: [1-(B-C)/S] ×100, where B represents represent the difference in the absorbance of the test sample. Kojic acid was used as references which are well-known tyrosinase inhibitor.

B. Measurement of melanin secretion in B16F10 melanoma cells

Extracellular melanin release was measured as previously described [4]. Briefly, B16F10 cells were incubated at a density of 1.5 × 105 cells in six-well plates overnight. 3-isobutyl-1-methylxanthine (IBMX) (100 μM) was then added and cells were treated with increasing concentrations of *Eruca sativa* extract in phenol red free DMEM for 3 days. 200 μl aliquots of media were then placed in 96-well plates and optical densities (OD) were measured at 405 nm using an ELISA reader (Beckman, Brea, CA, USA). Melanin productions were expressed as percentages of those of untreated controls.

III. RESULTS

To evaluate the direct effect of *Eruca sativa* extract on tyrosinase activity, mushroom tyrosinase assay was performed. *Eruca sativa* extract had inhibitory effect on tyrosinase activity, and the effect was not significantly low compared to kojic acid as a positive control (Fig. 1).

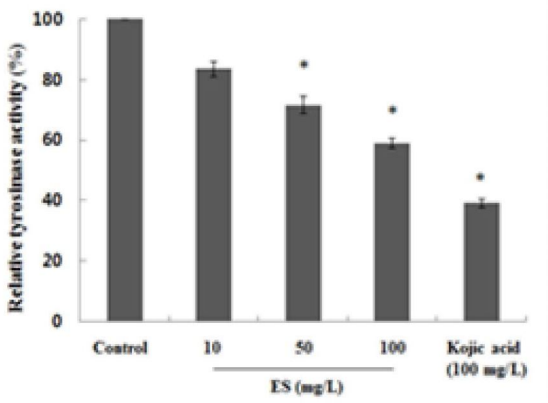

Fig. 1. Inhibitory effect of *Eruca sativa* extract (ES) on tyrosinase. The tyrosinase activity assay was performed with mushroom tyrosinase. Values are presented as mean ± SEM. Differences were considered statistically significant when *p< 0.05.

In addition, we treated B16F10 melanoma cells with *Eruca sativa* extract to determine whether it has a cytotoxic effect. Cell viability was determined using MTS assays. The results showed that *Eruca sativa* extract was not cytotoxic at B16F10 cells in the concentration range. Also, cells were exposed to *Eruca sativa* extract in the presence of IBMX for 3

days, and extracellular melanin release was measured. As shown in Figure 2, *Eruca sativa* extract reduced IBMX-induced melanin release in a dose-dependent manner.

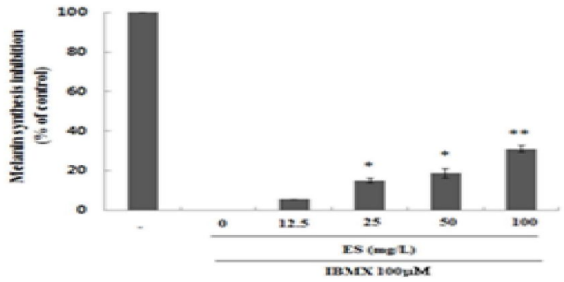

Fig. 2. Anti-melanogenic effects of *Eruca sativa* extract (ES). Cells were treated with 100μM IBMX in presence or absence of ES at the indicated concentration for 2 days.Values are presented as mean ± SEM. Differences were considered statistically significant when *p< 0.05, **p< 0.01.

Also, the treatment of *Eruca sativa* extract suppressed the protein expression of tyrosinase according to concentration (Fig. 3).

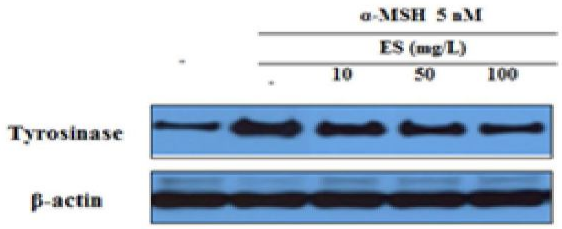

Fig. 3. Effect of *Eruca sativa* extract (ES) on expression of tyrosinase. β-actin was used as an internal standard.

CONCLUSION

These findings suggest that *Eruca sativa* extract inhibits the melanin synthesis by suppressing intracellular tyrosinase expression and direct inhibition of tyrosinase activity simultaneously.

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