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Plasmid DNA protective effect and antioxidative activity of enzymatic hydrolysates from *Umbilicaria esculenta*

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석이버섯으로부터 효소적 가수분해물의 항산화 활성 및 DNA 보호효과

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Objectives

Umbilicaria esculenta a foliose type macrolichen, has been regarded as an edible mushroom and used for a traditional food or a medicine in the Far East such as Korea, Japan and China. Extracts or prescriptions of *Umbilicaria esculenta* were known to have effects on treating many kinds of inflammation, bleeding and poisoning. It was also reported that *Umbilicaria esculenta* had activities on free radical scavenging. These results, indicate that enzymatic extracts of *Umbilicaria esculenta* possess potent antioxidative activity.

Materials and Methods

○ Materials

Umbilicaria esculenta was sourced from a local market (Chungju, Korea). In addition PC-12 Cells was obtained from Pukyong National University.

○ Methods

Free radical scavenging activity DPPH radical - A sample solution of 30 μL of each enzymatic extracts, was added to 30 μL of DPPH (30 μM) in methanol solution. After mixing vigorously for 10 sec, the solution was then transferred into a 100 μL Teflon capillary tube, and the scavenging activity of each enzymatic extract on DPPH radical was measured using a JES-FA ESR spectrometer (Jeol Ltd., Tokyo, Japan).

Alkyl radical - Alkyl radicals were generated by AAPH. The phosphate-buffered saline (PBS, pH 7.4) reaction mixtures containing 10 mM AAPH, 10 mM 4-POBN, and indicated concentrations of tested samples were incubated at 37℃ in a water bath for 30 min and then transferred to a 100 μL teflon capillary tube. The spin adduct was recorded on an ESR spectrometer.

DNA protective effects The protective effects of the enzymatic extracts on DNA damage induced by H_2O_2, the reaction was conducted in an Eppendorf tube at a total volume of 13 μL containing 0.5 μL of pBR 322 DNA in 3 μL of 50 mM phosphate buffer (pH 7.4), 3 μL of 2 mM $FeSO_4$ and 2 μL of the enzymatic extracts at various concentrations. Then 4 μL of 30% H_2O_2 was added, and the mixture was incubated at 37℃ for 1 h. The mixture was subjected to 0.8% agarose gel electrophoresis.

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Results

The *Umbilicaria esculenta* were enzymatically hydrolyzed by 7 carbohydrases (Dextrozyme, AMG, Promozyme, Maltogenase, Termamyl, Viscozyme, and Celluclast). The DPPH radical scavenging activity of Termamyl extracts was the highest, and the IC_{50} value was 562 μg/mL. The Viscozyme extracts showed the highest alkyl radical scavenging activity, and the IC_{50} value was 90 μg/mL. In addition, the Viscozyme extracts protective effects of pBR 322 plasmid DNA against H_2O_2-induced oxidative damage.

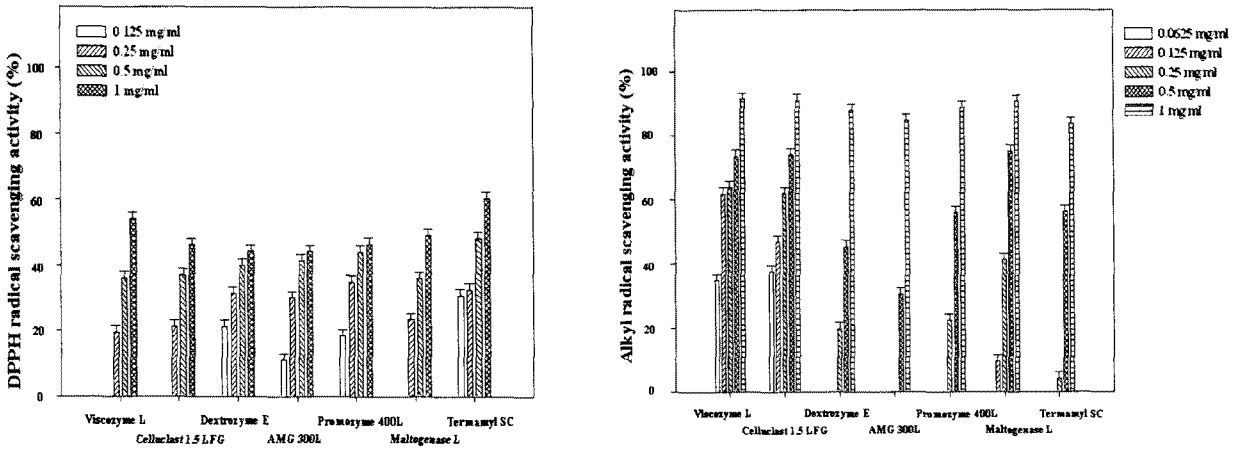

Fig 1. DPPH (left) and alkyl (right) radical scavenging activity of various enzymatic extracts by carbohydratic hydrolysis from *Umbilicaria esculenta*

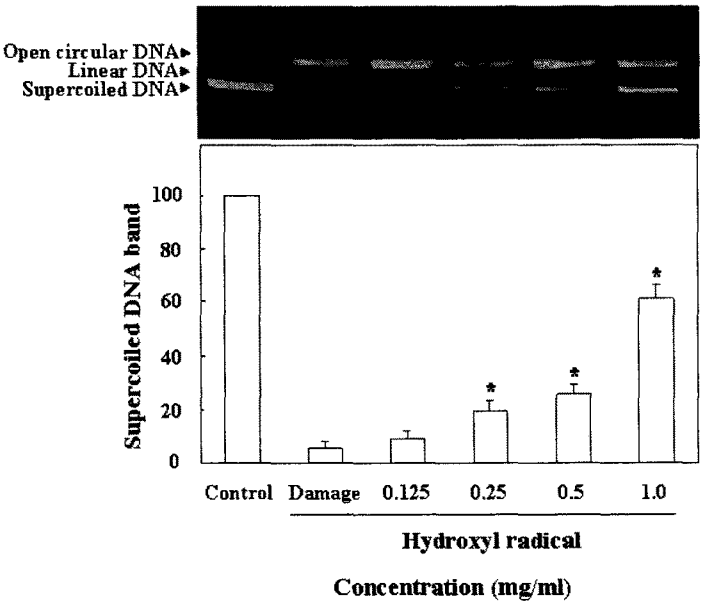

Fig 2. Agarose gel electrophoretic patterns of plasmid DNA breaks by ·OH generated from a Fenton reaction in the presence of Viscozyme hydrolysates from *Umbilicaria esculenta*