

Protective Effect of isolated fucoidan, sulfated polysaccharide from *Undaria pinnatifide* on connective tissue proteolysis

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미역 포자엽으로부터 황함유 다당체 fucoidan의 분리와
결합조직의 파괴에 대한 보호효과
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Objective

- Comparison of fucoidan content between seaweeds
- Isolation and Purification of Fucoidan Using Enzyme Hydrolysis
- Identification of defense mechanism against UV-irradiation induced photoaging through inhibition of MMP-1 production by fucoidan
- Inhibitory effect of 15kDa fucoidan in Human leukocyte elastase activity

Materials and Methods

○ **Materials**

Hizikia fusiformis(툫), *Sarassum fuvellum*(모자반), *Undaria pinnatifide*(미역포자엽), *Laminaria religiosa*, (다시마) → Comparison of fucoidan and alginate contents

○ **Methods**

1. Crude fucoidan fractionation

- *Undaria pinnatifide* (56kg) → Hydrolysis [cellulase(500U/100g), pectinase(100U/100g) arylsulfatase(25U/100g)] → 2% CaCl₂ (separation of alginic acid) → cetylpyridinium chloride and ethanol precipitation → dialysis

2. Fucoidan Isoation

- Ion-exchange chromatography [DEAE-Sephadex A-25 (30×1.7cm, Pharmacia), NaCl 0-3.5M] → Dialysis [0.1 M pyridine acetate(pH 5-5.4), 3days]
- Carbohydrate analysis : high performance anion-exchange chromatography (Dubois et al., 1956)
- Sulfate analysis : gelatin/BaCl₂ method (Dodgson, 1961; Saito et al., 1968).
- Uronic acid content : carbazole method (Bitter and Muir, 1962)

3. UVB irradiation and Fucoidan treatment

4. Gelatinolytic activity test

5. Western blot (MMP, TIMP)

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6. *Ex vivo* HLE inhibition assay

Results

1. Comparison of fucoidan and alginate contents between seaweeds

Table 1. Yields of crude fucoidan and partially purified fucoidans extracted from brown seaweeds

Species	Crude fucoidan (% of brown seaweed)	Crude Alginate (% of brown seaweed)
돛(<i>Hizikia fusiformis</i>)	4.11±0.41	24.75±1.62
모자반(<i>Sarassum fuvellum</i>)	4.23±0.72	34.48±1.43
미역포자엽 (<i>Undaria pinnatifide</i>)	13.11±0.69	21.65±1.84
다시마(<i>Laminaria religiosa</i>)	4.73±0.45	26.16±1.54



2. Purification of Fucoidan and Photoprotective effect of Fucoidan against UV-B

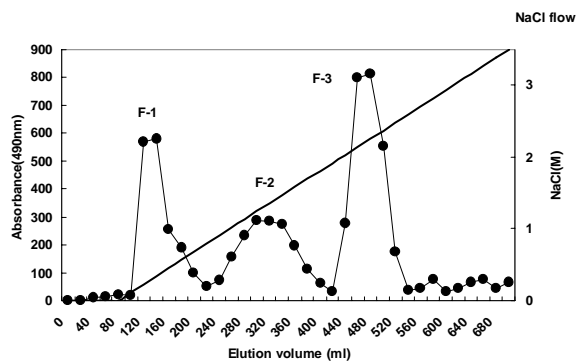


Figure 1. Fractionation of crude fucoidan isolated from the brown alga *Undaria pinnatifide* on a DEAE-Sephadex A-25 column

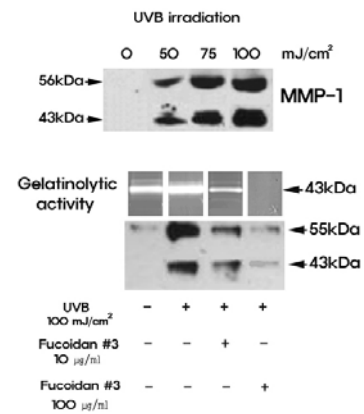


Figure 3. Inhibition of UV-induced MMP-1 expression by isolated fucoidan F-3 from *Undaria pinnatifide* in human fibroblast (HS68).

3. *Ex vivo* HLE inhibition assay

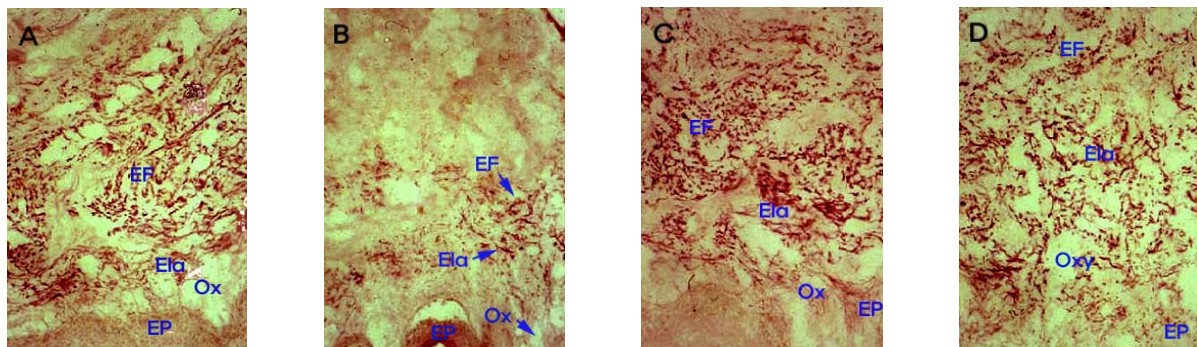


Figure 11. Fucoidan inhibits dermal elastic fibers hydrolysis mediated HLE by histological section of mid dermal elastic fiber serial section. A, control; B, HLE 2 µg; C, HLE 2 µg with fucoidan 10 µg/ml; D, HLE 2 µg with fucoidan 100 µg/ml; EP, epithelium; Oxy, oxytalan; Ela, elaunin fiber(elasticin); EF, elastic fiber,