

## Development of health functional food and functional cosmetics from natural products

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### Abstract

*Vaccinium uliginosum L.* (VU) is a flowering plant in the genus *Vaccinium* has berry fruit. This study was performed to investigate the effect of water extract of *Vaccinium uliginosum L.* and fractions on inhibition of melanogenesis and wrinkle formation. One hundred grams of the *Vaccinium uliginosum L.* was extracted with 2,000 mL of water (90 °C, 16h, 2 times). The water extracts were lyophilized and stored at 4 °C until used. *Vaccinium uliginosum L.* extracts showed scavenger activities on D PPH radical, superoxide anion radical, hydroxyl radical, hydrogen peroxide and singlet oxygen radical, dose dependently. And VU extract and fractions reduced melanin contents on B16F10 melanoma and inhibited the expression of melanogenesis-related proteins, tyrosinase, tyrosinase-related protein (TRP-1) and dopachrome tautomerase (Dct, TRP-2).

Moreover VU extract and fractions stimulated procollagen production and inhibited MMP-1 production in human fibroblast. And it decreased degree of wrinkle formation in hairless mouse skin that induced by UVB irradiation for 9 weeks.

From the above results, it is possible that *Vaccinium uliginosum L.* may be developed to be the health functional food and functional cosmetics that have anti-melanogenesis and anti-wrinkle effect.

## *Vaccinium uliginosum* L.



들쭉 (*Vaccinium uliginosum*)

철쭉과 (*Ericaceae*)

들쭉나무의 열매

천연기념물 461호

자생지 : 백두산, 일본, 유럽일대의  
고원, 한대에 분포

◆ 효능 : 모세혈관 강화작용, 혈당 저하, 대장암 예방, 피로회복, 정혈작용, 이뇨작용, 정력증강, 해열작용, 원기재고, 류마티스 관절염 및 통풍에 효과

◆ 효능성분 : 플라보노이드, 안토시아닌, 우르솔산, 사과산, 전화당, 자당

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## Substances

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< *Vaccinium uliginosum* L. >



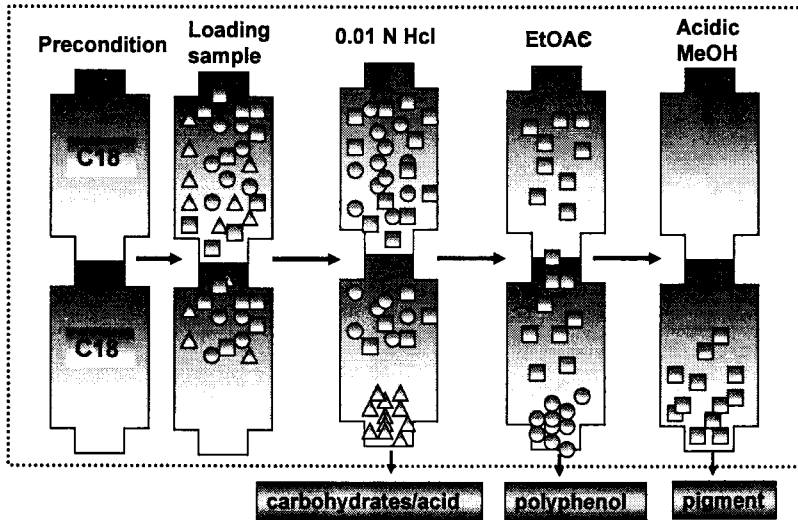
extracted with 2000 mL of  
water (90 °C, 16h, 2times)



The water extracts were  
lyophilized and stored at 4 °C  
until used.

## Isolation of fraction of *Vaccinium uliginosum L.*

Method of Solid-phase extraction by D.O Kim



Ref. *Current Protocols in Food Analytical Chemistry* (2002) / 1.2.1- / 1.2.12

## Anti-oxidative effect of *Vaccinium uliginosum L.* extract and fractions

- Free radical scavenger assay of *Vaccinium uliginosum L.* extract and fractions

## 1. Reactive oxygen species (ROS) 소거능

### 1-1. DPPH radical 소거능 측정

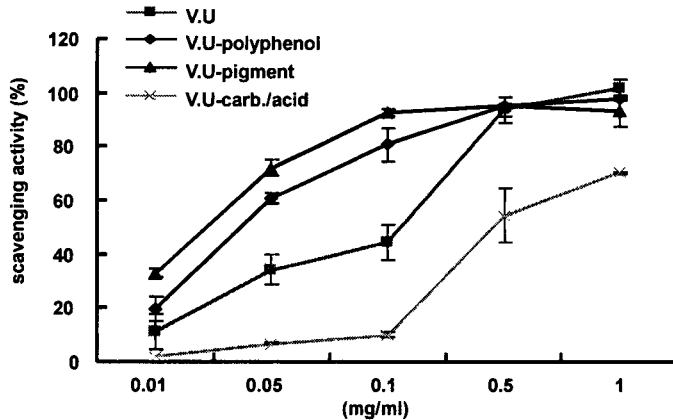


Fig. 1. DPPH radical scavenger activity of *Vaccinium uliginosum L.* extract and fractions.

(V = Extracts of *Vaccinium uliginosum L.*)

Letters (alphabets) different superscripts are significantly different ( $p < 0.05$ ) among the groups by Duncan's multiple range test.

### 1-2. Superoxide radical 소거능 측정

#### Non-enzymatic system

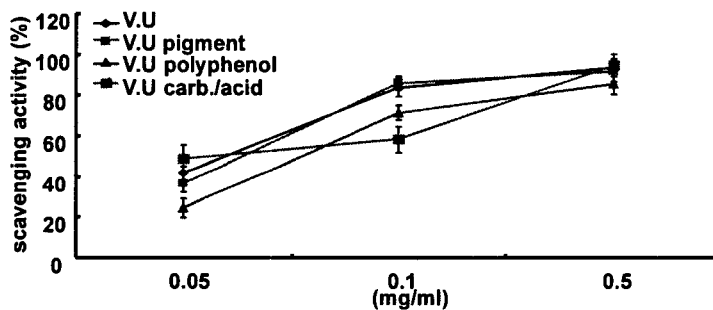


Fig. 2. Superoxide radical scavenger activity of *Vaccinium uliginosum L.* extract and fractions in NADH/PMS system.

(V = Extracts of *Vaccinium uliginosum L.*)

Letters (alphabets) different superscripts are significantly different ( $p < 0.05$ ) among the groups by Duncan's multiple range test

1-2. Superoxide radical 소거능 측정  
Enzymatic system

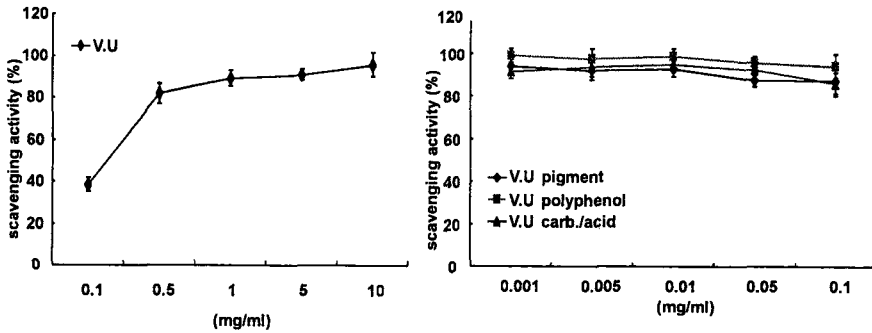


Fig. 3. Superoxide radical scavenger activity of *Vaccinium uliginosum L.* extract and fractions in xanthine-xanthine oxidase system.

(V = Extracts of *Vaccinium uliginosum L.*)

Letters (alphabets) different superscripts are significantly different ( $p < 0.05$ ) among the groups by Duncan's multiple range test

1-3. Hydroxyl radical 소거능 측정

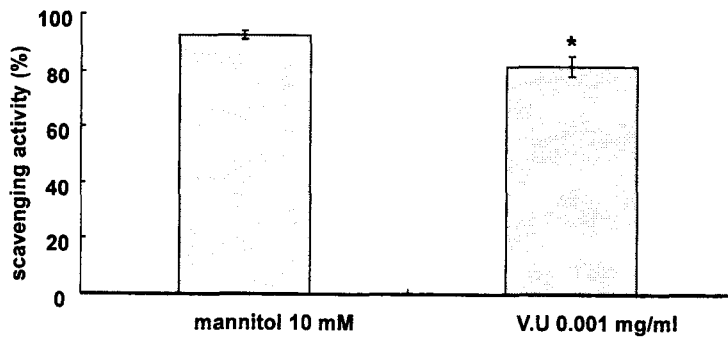


Fig. 4. Hydroxyl radical scavenger activity of *Vaccinium uliginosum L.* extract.

(V = Extracts of *Vaccinium uliginosum L.*)

\* different superscripts are significantly different ( $p < 0.05$ ) among the groups by Duncan's multiple range test.

#### 1-4. Singlet oxygen radical 소거능 측정

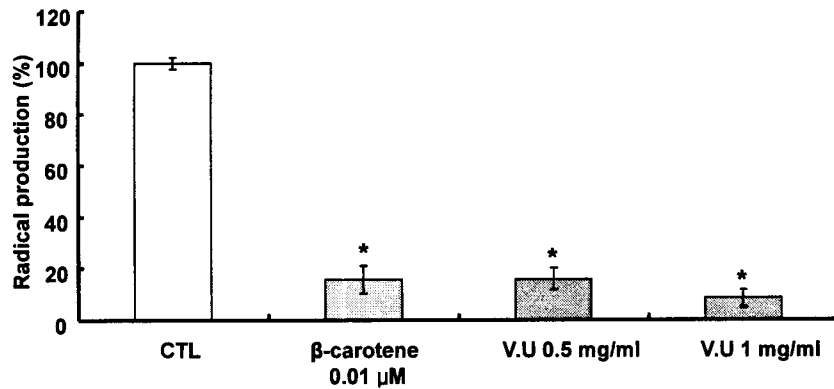


Fig. 5. Singlet oxygen radical scavenger activity of *Vaccinium uliginosum L.* extract.

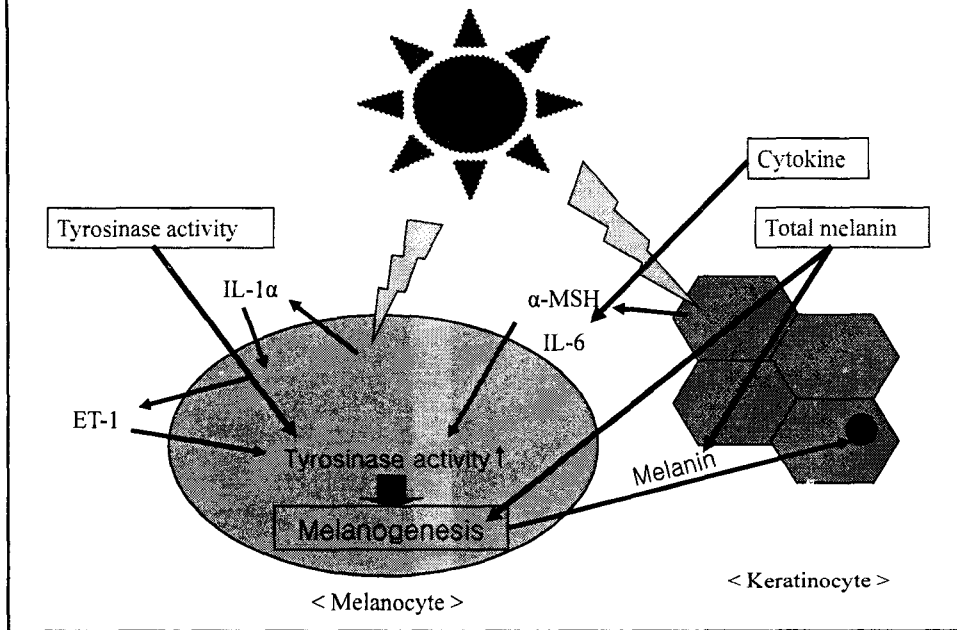
(V.U = Extracts of *Vaccinium uliginosum L.*)

\* different superscripts are significantly different ( $p < 0.05$ ) among the groups by Duncan's multiple range test.

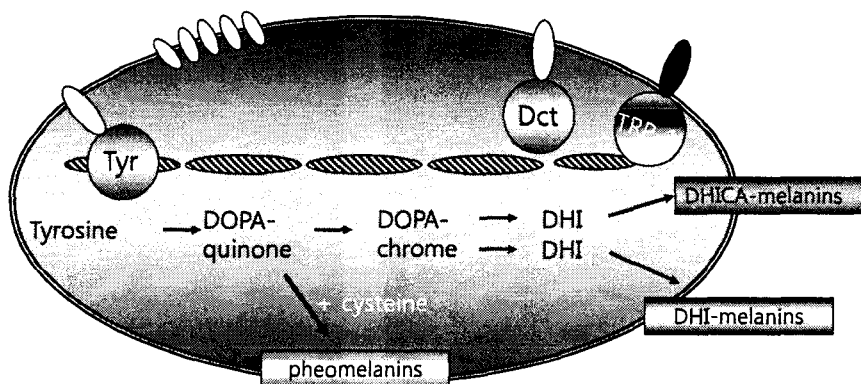
## Anti-melanogenesis effects of *Vaccinium uliginosum L.* extract and fractions

- The tyrosinase inhibitory activity
- The measurement of melanin contents on B16F10 melanoma cell
- The expression of melanogenesis-related protein on B16F10 cell

## Mechanism of melanogenesis



## Scheme of melanosome maturation



Tyr=tyrosinase, TRP=tyrosinase-related protein, Dct=dopachrome tautomerase,

DHI=5,6-dihydroxyindole DHICA=5,6-dihydroxyindole-2-carboxylic acid (DHI/DHICA= unit of eumelanin)

## Total melanin contents

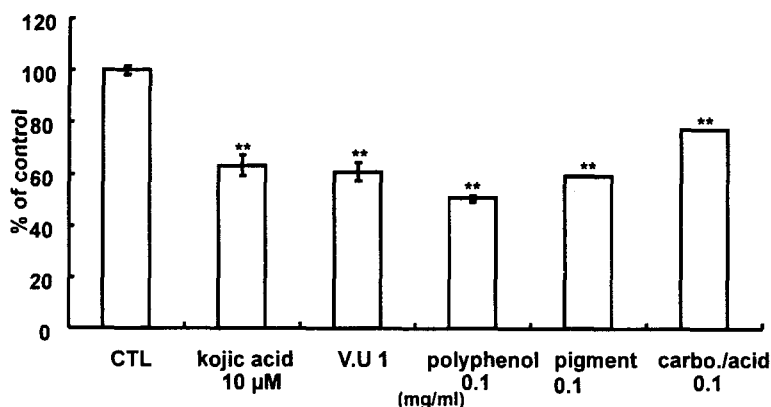


Fig. 7. Total melanin concentration in B 16 melanoma cells treated with extracts of *Vaccinium uliginosum L.* and fractions.

(V.U = Extracts of *Vaccinium uliginosum L.* extract)

Letters (alphabets) different superscripts are significantly different ( $p < 0.01$ ) among the groups by Duncan's multiple range test.

## Tyrosinase inhibition activity

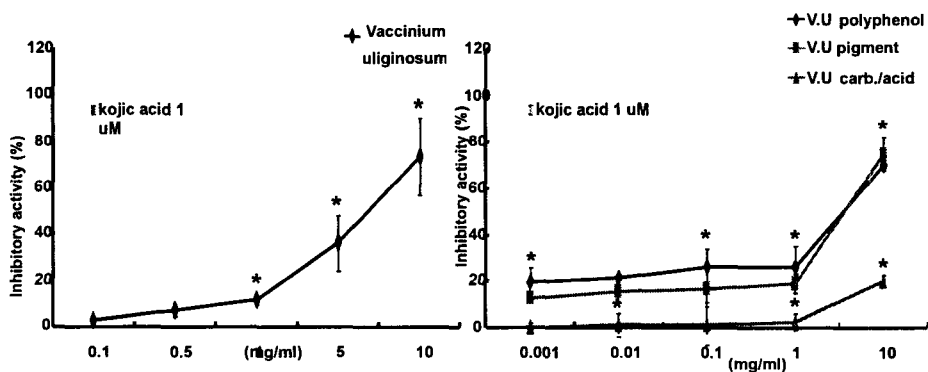


Fig. 6. Tyrosinase inhibition activity assay of *Vaccinium uliginosum L.* extract and fractions.

(V.U = Extracts of *Vaccinium uliginosum L.*)

Letters (alphabets) different superscripts are significantly different ( $p < 0.05$ ) among the groups by Duncan's multiple range test.



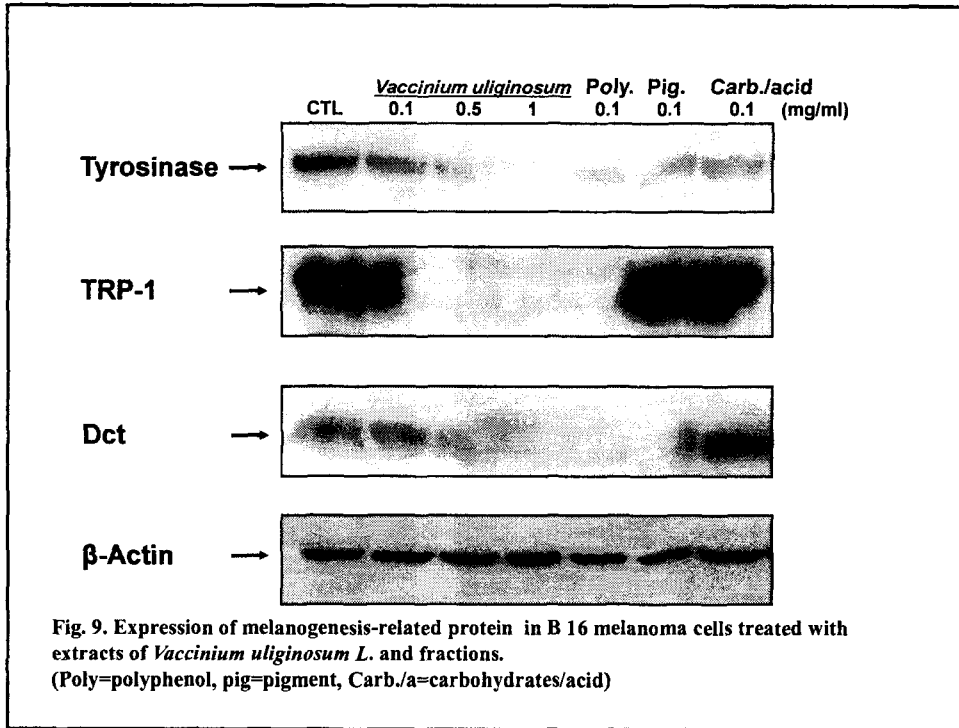
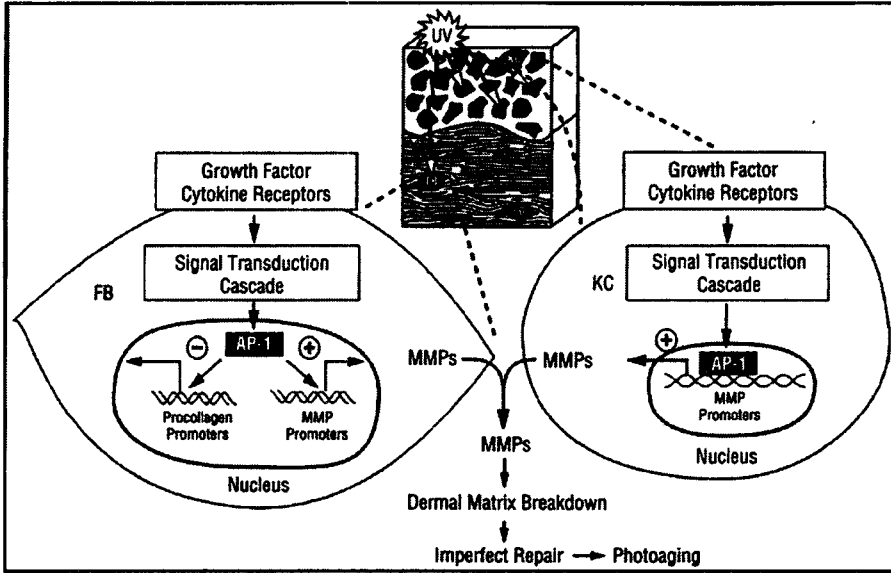


Fig. 9. Expression of melanogenesis-related protein in B 16 melanoma cells treated with extracts of *Vaccinium uliginosum* L. and fractions. (Poly=polyphenol, pig=pigment, Carb./a=carbohydrates/acid)

### *In vitro* anti-wrinkle effects of *Vaccinium uliginosum* L. extract and fractions

- The quantitative analysis of type I collagen synthesis
- The quantitative analysis of MMP-1 production
- The animal test

## Mechanism of wrinkle formation by photoaging



## Type-1 Procollagen

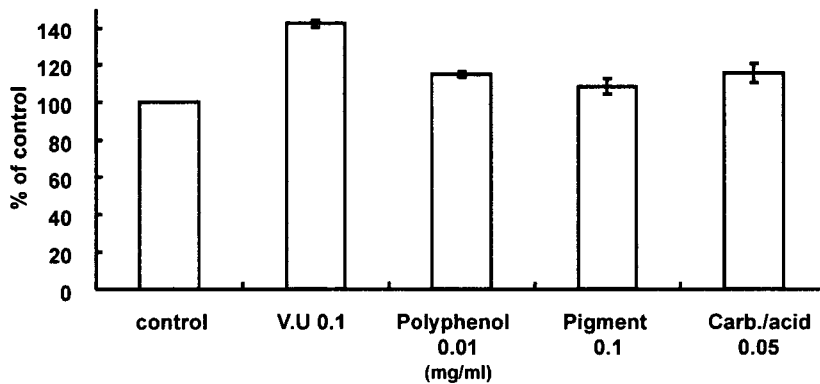


Fig.10. Type-1 Procollagen concentration (ng/mL) of human fibroblast cells treated with extracts of *Vaccinium uliginosum L.* and fractions.

(V = Extracts of *Vaccinium uliginosum L.*)

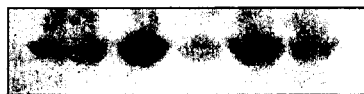
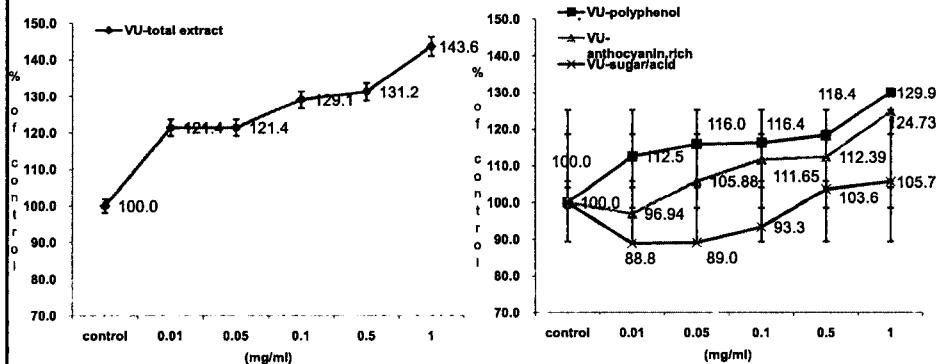
# Matrix Metalloproteinase(MMP-1)

**Table 1. Matrix Metalloproteinase-1(MMP-1) concentration(ng/mL) of human fibroblast cells treated with extracts of *Vaccinium uliginosum L.* extract.**

MMP-1 Conc.(ng/mL)	V.U.
Conc. Of Sample(mg/mL)	
Control	37.2 ± 4.7 <sup>1)</sup>
0.001	32.8 ± 3.1
0.005	27.1 ± 5.5
0.01	25.5 ± 1.3

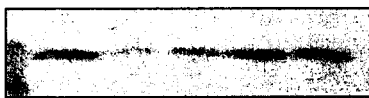
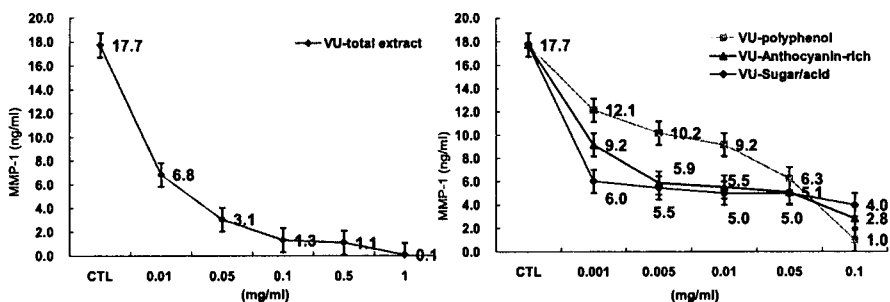
1) Mean ± S.D.

# Type-1 procollagen



CTL VU Anthoc polyphs Su/a  
(mg/ml) 1.0 0.1 0.1 0.1

## Matrix Metalloproteinase(MMP-1)

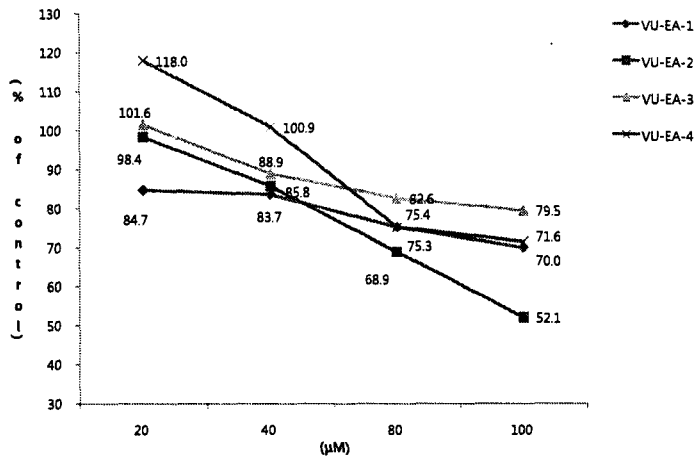


CTL VU polyphenol Anthocyanin Su/a  
(mg/ml) 1.0 0.1 0.1 0.1

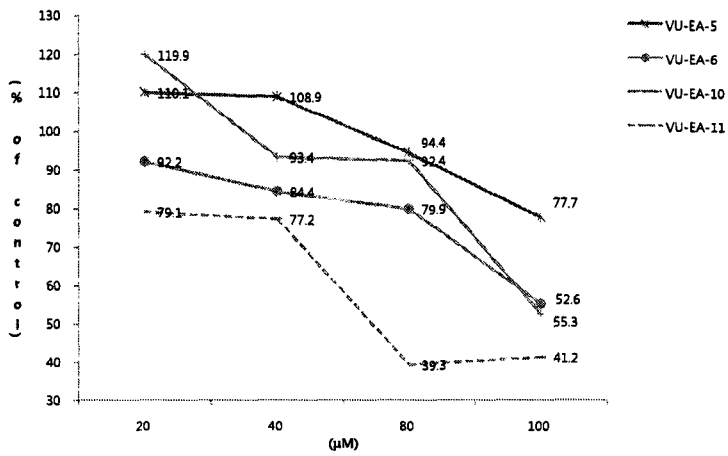
## Anti-melanogenesis effect of purified single compounds

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### Total melanin contents



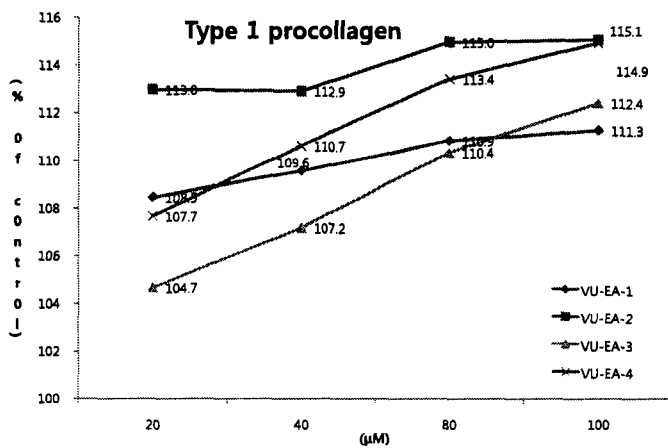
### Total melanin contents



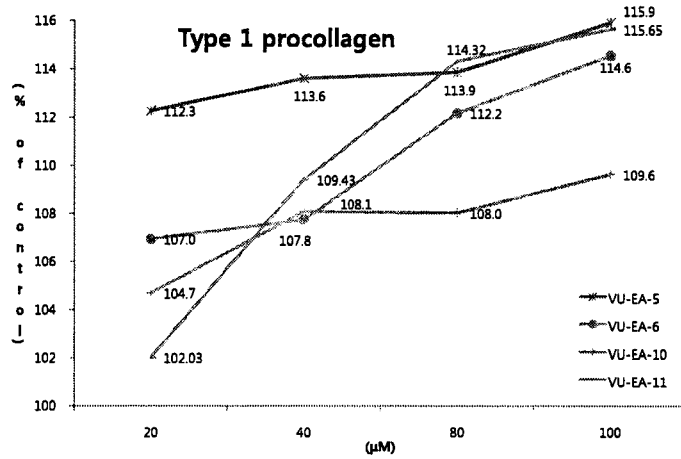
# Anti-wrinkle effect of purified single compounds

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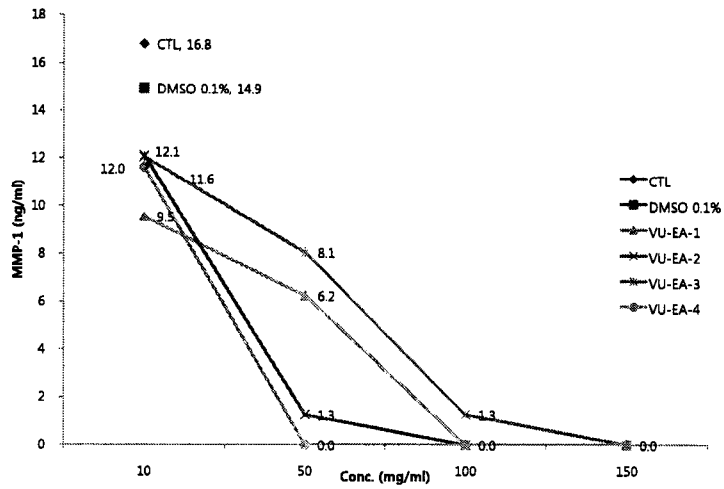
## Procollagen-1



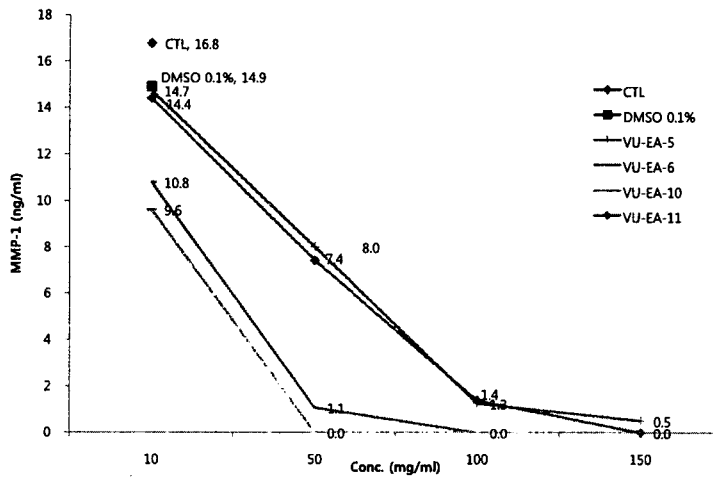
## Procollagen-1



## Matrix Metalloproteinase(MMP-1)



## Matrix Metalloproteinase(MMP-1)



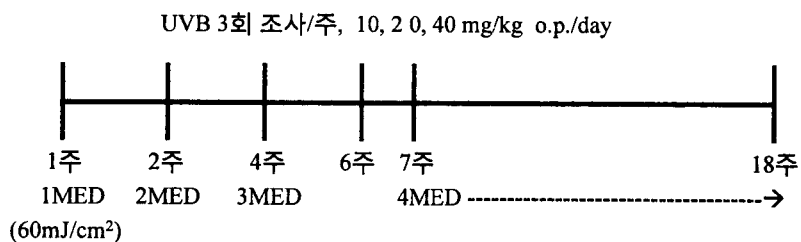
**Anti-wrinkle effects of (hairless mouse)  
*Vaccinium uliginosum L.* extract**

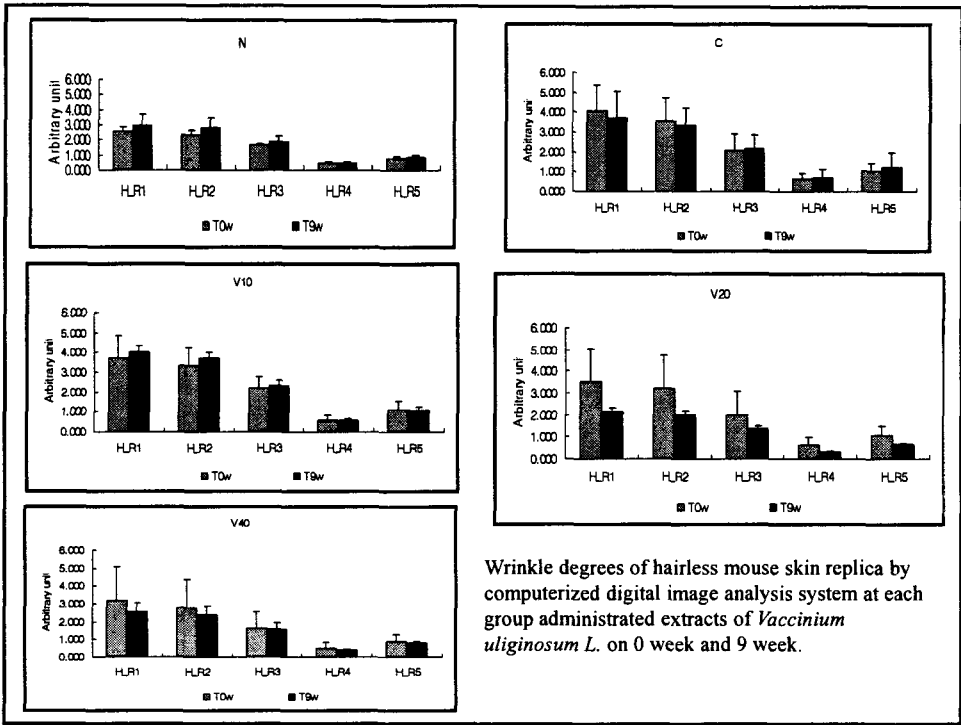
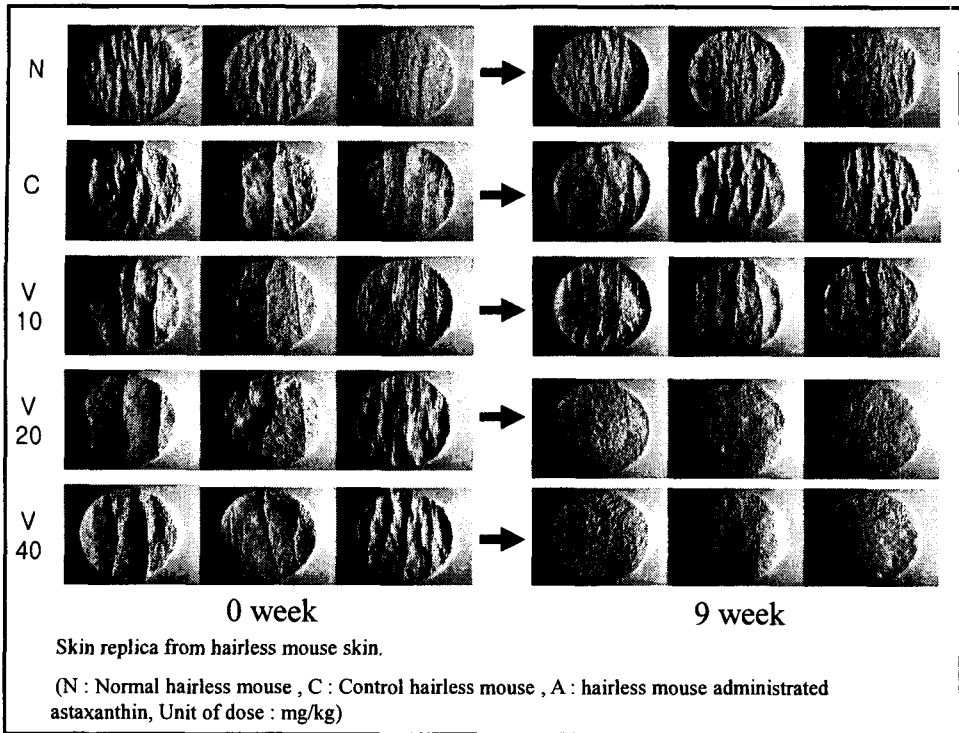


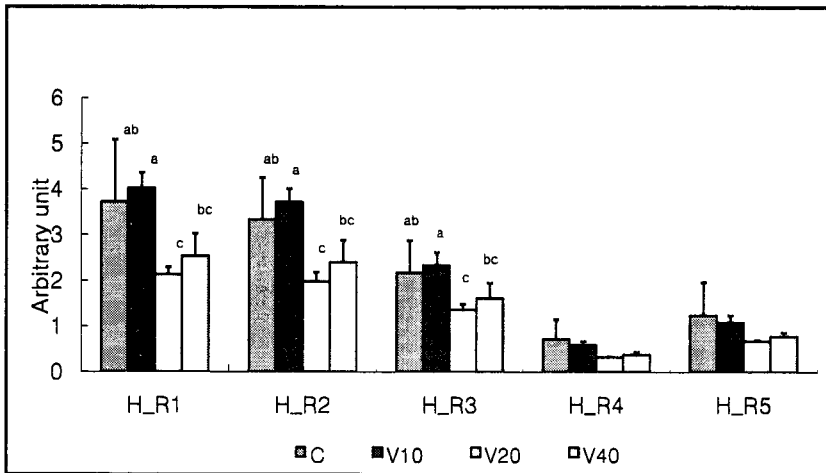
## Oral administration

<b>No UVB</b>	<b>Normal</b>
<b>UVB 조사군</b>	<b>UVB + Control</b>
	<b>UVB + <i>Vaccinium uliginosum</i> 10mg/kg</b>
	<b>UVB + <i>Vaccinium uliginosum</i> 20mg/kg</b>
	<b>UVB + <i>Vaccinium uliginosum</i> 40mg/kg</b>

## Oral administration schedule







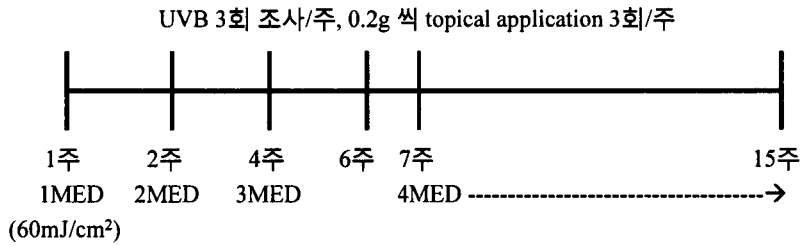
Wrinkle degrees of hairless mouse administrated extracts of *Vaccinium uliginosum* L. by computerized digital image analysis system on 9 week.

Letters (alphabets) different superscripts are significantly different ( $p < 0.05$ ) among the groups by Duncan's multiple range test.

## Topical application

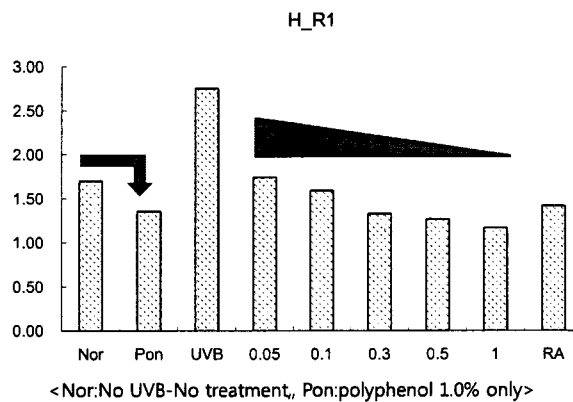
<b>No UVB</b>	<b>Normal</b>
	<b>Positive control (retinol acid 2500IU)</b>
	<b>Polyphenol 1.0%</b>
<b>UVB 조사군</b>	<b>UVB-induced wrinkle formation</b>
	<b>UVB + polyphenol 0.05%(v/v)</b>
	<b>UVB + polyphenol 0.1%(v/v)</b>
	<b>UVB + polyphenol 0.3%(v/v)</b>
	<b>UVB + polyphenol 0.5%(v/v)</b>
	<b>UVB + polyphenol 1.0%(v/v)</b>
	<b>Vehicle (cream only)</b>

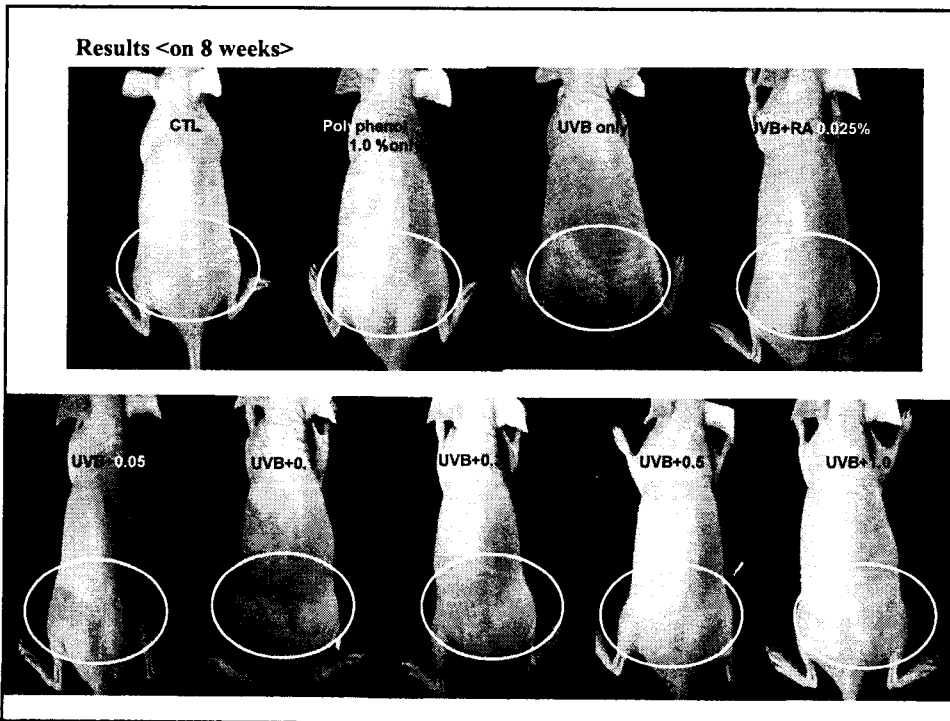
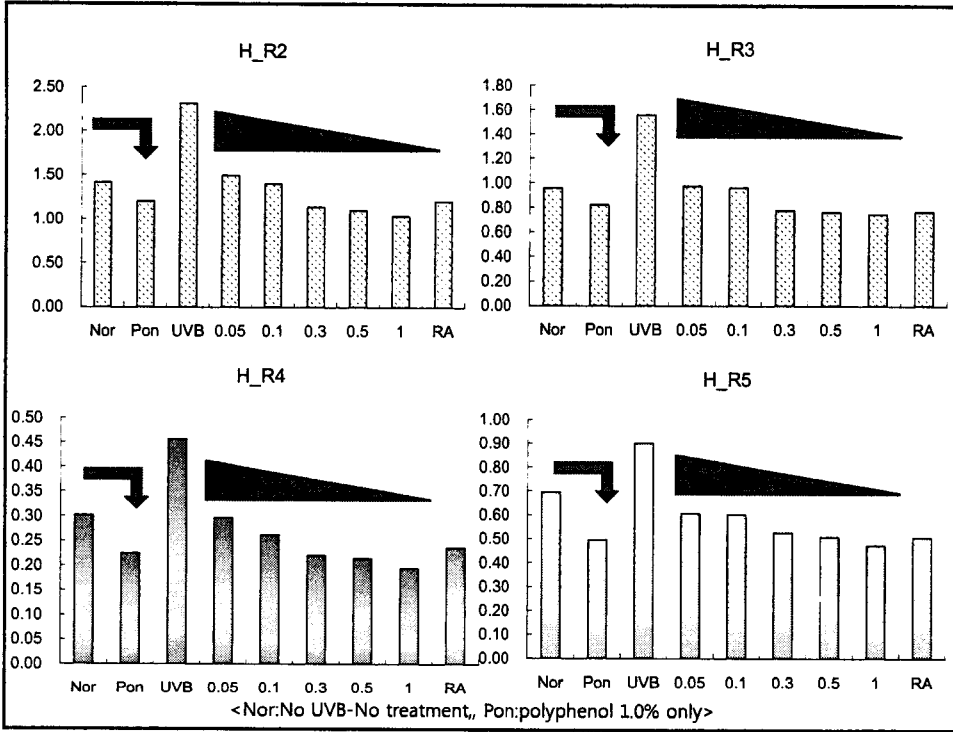
# Topical application schedule



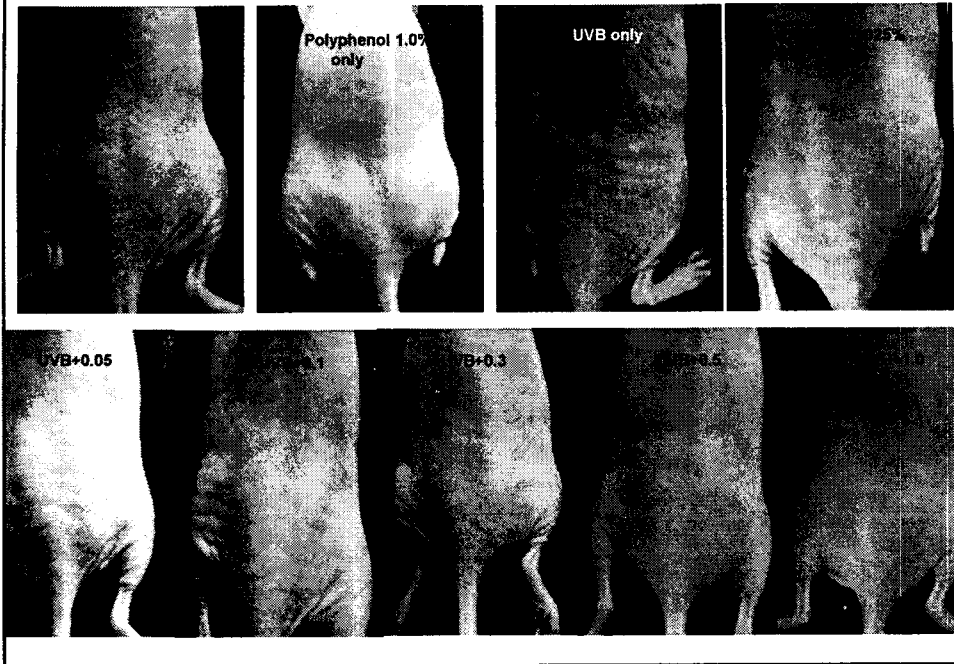
## Results of standard sample-Topical application

- ◆ The data analyzed into 5 different factors of skin fold thickness and roughness.
  - H\_R1~3 means the depth of the wrinkle.
  - H\_R4 means the roughness of the skin.
  - H\_R5 means the depth of the narrow wrinkle.
- ◆ The lower value of data means the thin and narrow wrinkle was formed and the skin surface is flat.





Results <on 15 weeks>



## Summary

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