

**Development of *Vaccinium uliginosum* L.
extracts for whitening & anti-wrinkle
functional food**

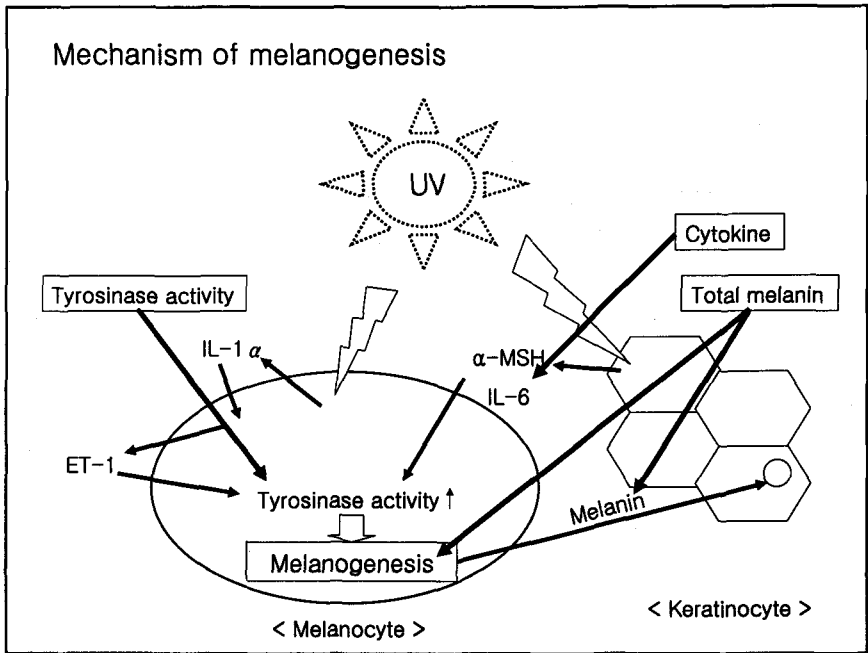
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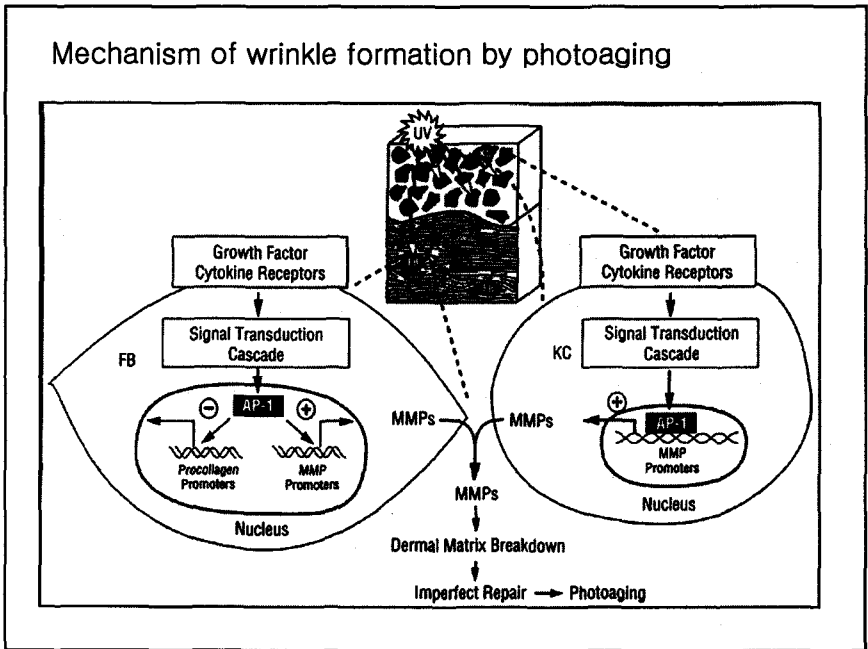
Introduction

- **Aging or aged society**
- **Skin whitening : mainly cosmetics (kojic acid)**
- **Anti-wrinkle**
: mainly cosmetics (Vit A derivatives, Vit E)
plastic operation
- **Well being trend : quality of life**

Mechanism of melanogenesis



Mechanism of wrinkle formation by photoaging



Substances

< *Vaccinium uliginosum* L. >



Methods

In vitro Anti-oxidative effect

Free radical scavenger assay of *Vaccinium uliginosum* L. extracts

1. DPPH radical
2. Superoxide anion radical
NADH/PMS system
Xanthine-Xanthine oxidase system
3. Hydroxyl radical
4. Singlet oxygen radical

Free radical scavenger assay of *Vaccinium uliginosum* L. extracts in human keratinocyte

1. Superoxide anion radical
2. Hydroxyl radical
3. Hydrogen peroxide
4. Singlet oxygen radical

***In vitro* Anti-melanogenesis effect**

1. Tyrosinase activity assay
2. Total melanin in B16 melanoma cells
3. Total melanin in B16 melanoma cells after UV B irradiation

***In vitro* Anti-wrinkle effect**

1. Type-1 procollagen in human dermal fibroblast cells
2. Matrix metalloproteinase-1(MMP-1 ; collagenase) in human dermal fibroblast cells
3. IL-6 in human keratinocyte after UV B irradiation
4. Type-1 procollagen in human dermal fibroblast cells after UV B irradiation
5. Matrix metalloproteinase-1(MMP-1 ; collagenase) in human dermal fibroblast cells after UV B irradiation

Anti-oxidant effect of *Vaccinium uliginosum* L.

- **Free radical scavenger assay of *Vaccinium uliginosum* L. extracts**

DPPH radical scavenger activity

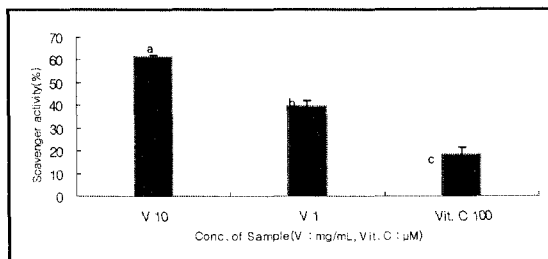


Fig. 1. DPPH radical scavenger activity of *Vaccinium uliginosum L.*

(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.

Superoxide radical scavenger activity

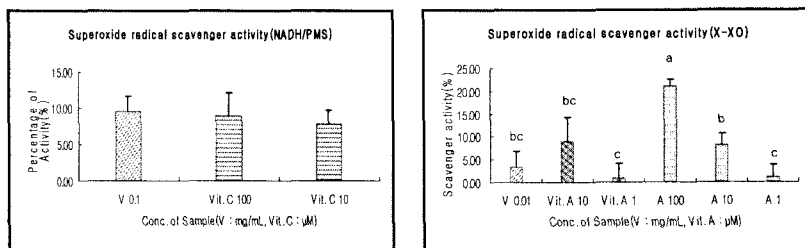


Fig. 2. Superoxide radical scavenger activity of *Vaccinium uliginosum L.* in NADH/PMS system and xanthine-xanthine oxidase system.

(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

Hydroxyl radical scavenger activity

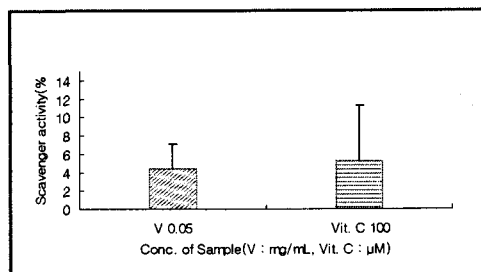


Fig. 3. Hydroxyl radical scavenger activity of *Vaccinium uliginosum L.*

(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.

Singlet oxygen radical scavenger activity

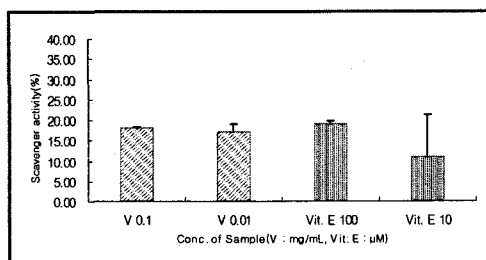


Fig. 4. Singlet oxygen radical scavenger activity of *Vaccinium uliginosum L.*

(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.

Anti-oxidant effect of *Vaccinium uliginosum* L.

- Free radical scavenger assay of *Vaccinium uliginosum* L. extracts in human keratinocyte

Superoxide radical scavenger activity

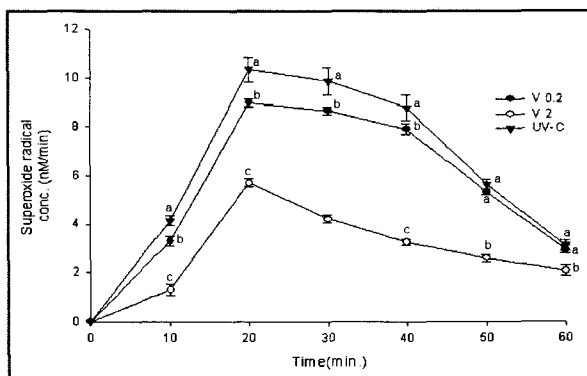


Fig. 5. Superoxide radical from keratinocyte treated with extracts of *Vaccinium uliginosum* L. after UV B irradiation.

(V = Keratinocyte treated with extracts of *Vaccinium uliginosum* L., UV-C = untreated keratinocyte, Concentration unit of extracts of *Vaccinium uliginosum* L. : mg/mL)

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

Hydroxyl radical scavenger activity

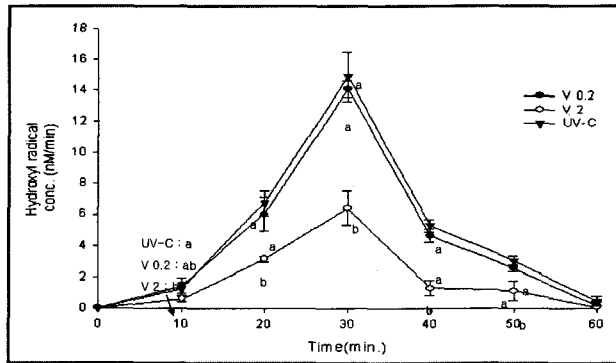


Fig. 6. Hydroxyl radical from keratinocyte treated with extracts of *Vaccinium uliginosum L.* after UV B irradiation.
 (V = Keratinocyte treated with extracts of *Vaccinium uliginosum L.*, UV-C = untreated keratinocyte, Concentration unit of extracts of *Vaccinium uliginosum L.* : mg/mL)
 Letters (alphabets) different superscripts are significantly different ($p < 0.05$) among the groups by Duncan's multiple range test.

Hydroxyl radical scavenger activity

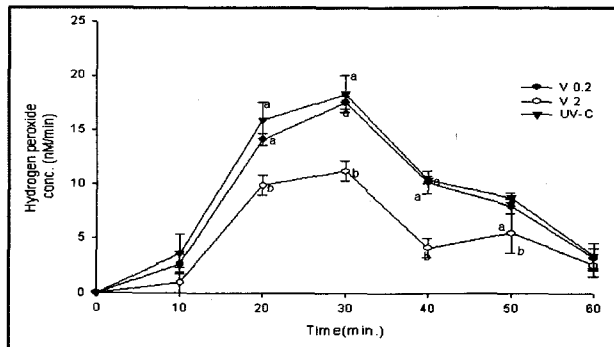


Fig. 7. Hydrogen peroxide from keratinocyte treated with extracts of *Vaccinium uliginosum L.* after UV B irradiation.
 (V = Keratinocyte treated with extracts of *Vaccinium uliginosum L.*, UV-C = untreated keratinocyte, Concentration unit of extracts of *Vaccinium uliginosum L.* : mg/mL)
 Letters (alphabets) different superscripts are significantly different ($p < 0.05$) among the groups by Duncan's multiple range test.

Singlet oxygen radical scavenger activity

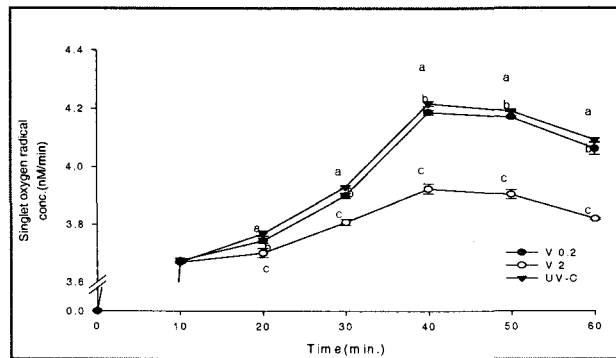


Fig. 8. Singlet oxygen radical from keratinocyte treated with extracts of *Vaccinium uliginosum L.* after UV B irradiation.

(V = Keratinocyte treated with extracts of *Vaccinium uliginosum L.*, UV-C = untreated keratinocyte, Concentration unit of extracts of *Vaccinium uliginosum L.* : mg/mL)

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

Anti-melanogenesis effects of *Vaccinium uliginosum L.*

Tyrosinase inhibition activity

Table 1. Tyrosinase inhibition activity assay

Inhibition rate(%) Conc. Of Sample(μ M)	Kojic acid	Inhibition rate(%) Conc. Of Sample(mg/mL)	V.U.
100	84.4 \pm 8.1	1	72.8 \pm 5.7
10	50.8 \pm 9.6	0.5	58.4 \pm 3.4
1	14.4 \pm 4.5	0.25	33.2 \pm 4.8
0.1	9.7 \pm 3.7	0.1	11.4 \pm 2.4

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

Total melanin

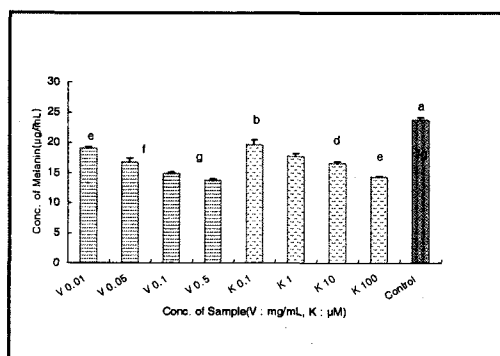


Fig. 9. Total melanin concentration in B 16 melanoma cells treated with extracts of *Vaccinium uliginosum L.*

(V = Extracts of *Vaccinium uliginosum L.*, K = Kojic acid)

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

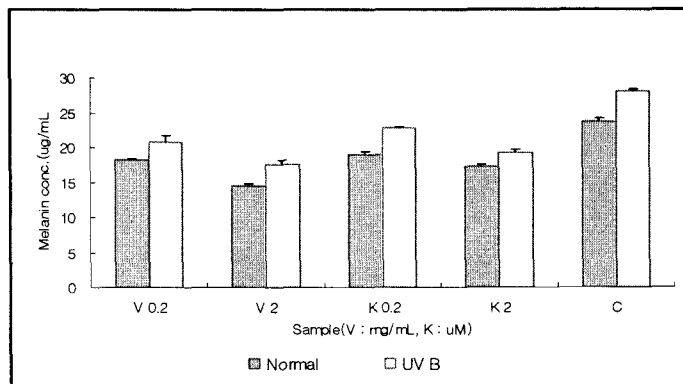


Fig. 10. Total melanin concentration in B 16 melanoma cells treated with extracts of *Vaccinium uliginosum L.* after UV B irradiation.

(V = Extracts of *Vaccinium uliginosum L.*, K = Kojic acid)

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

In vitro* anti-wrinkle effects of *Vaccinium uliginosum L.

Procollagen

Table 2. Procollagen concentration(ng/mL) of human fibroblast cells treated with extracts of *Vaccinium uliginosum L.*

Conc. Of Sample(mg/mL) \ Procollagen Conc.(ng/mL)	V.U.
Control	149.6 ± 25.4 ¹⁾
0.001	144.9 ± 21.8
0.005	155.1 ± 20.1
0.01	179.2 ± 31.1

1) Mean ± S.D.

Matrix Metalloproteinase(MMP-1)

Table 3. Matrix Metalloproteinase-1(MMP-1) concentration(ng/mL) of human fibroblast cells treated with extracts of *Vaccinium uliginosum L.*

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

1) Mean ± S.D.

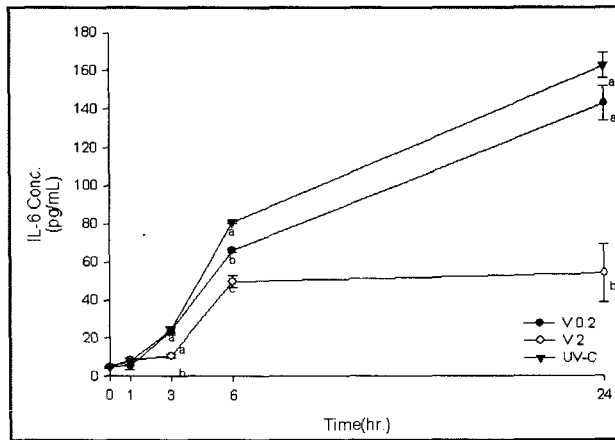


Fig. 11. Interleukin-6 release from keratinocyte treated with extracts of *Vaccinium uliginosum L.* after UV B irradiation.

(V = Keratinocyte treated with extracts of *Vaccinium uliginosum L.*, UV-C = untreated keratinocyte, Concentration unit of extracts of *Vaccinium uliginosum L.* : mg/mL)

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

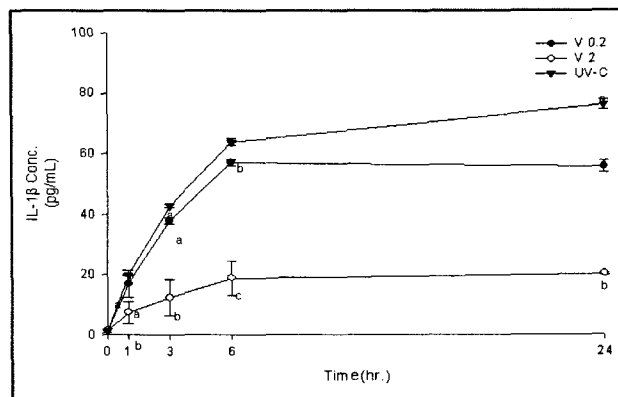
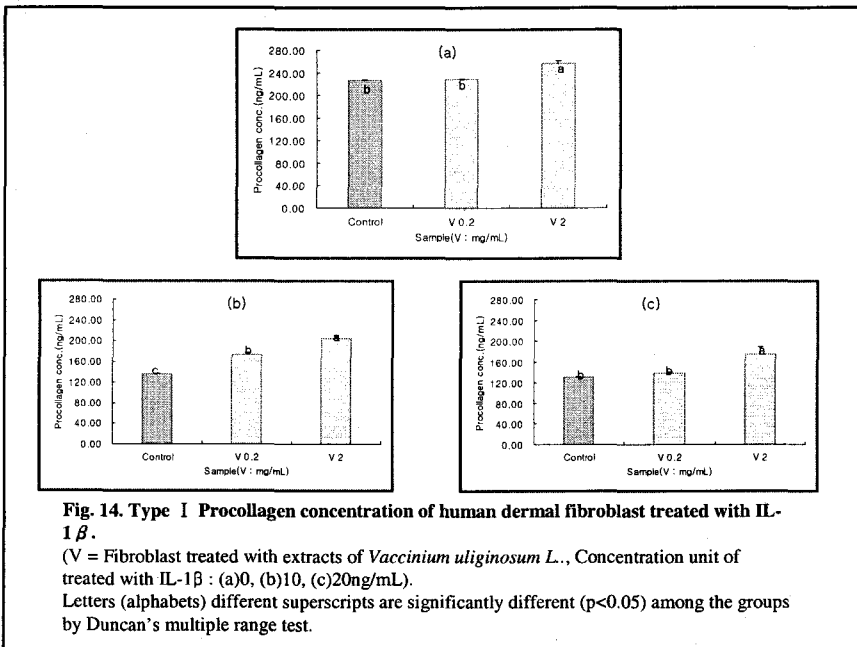
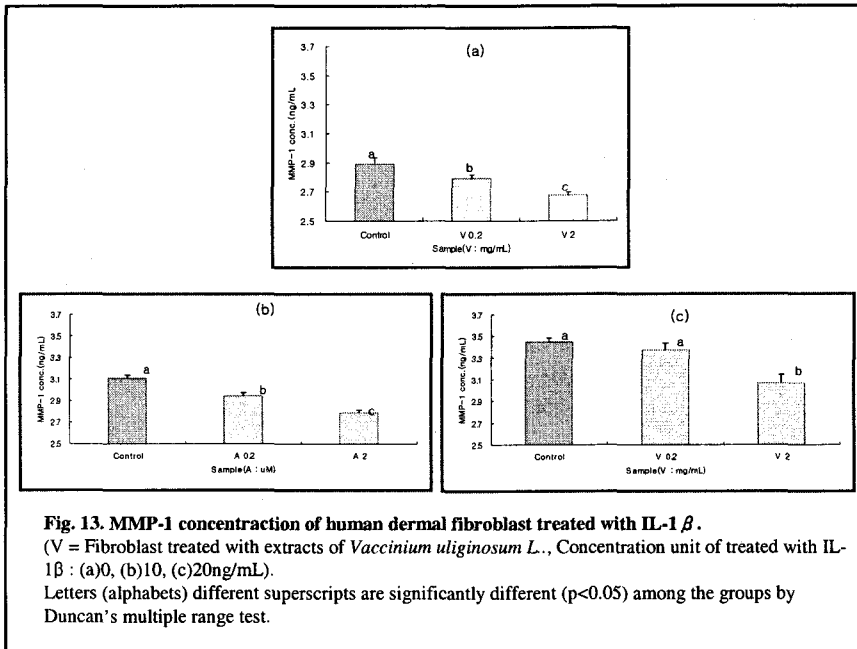


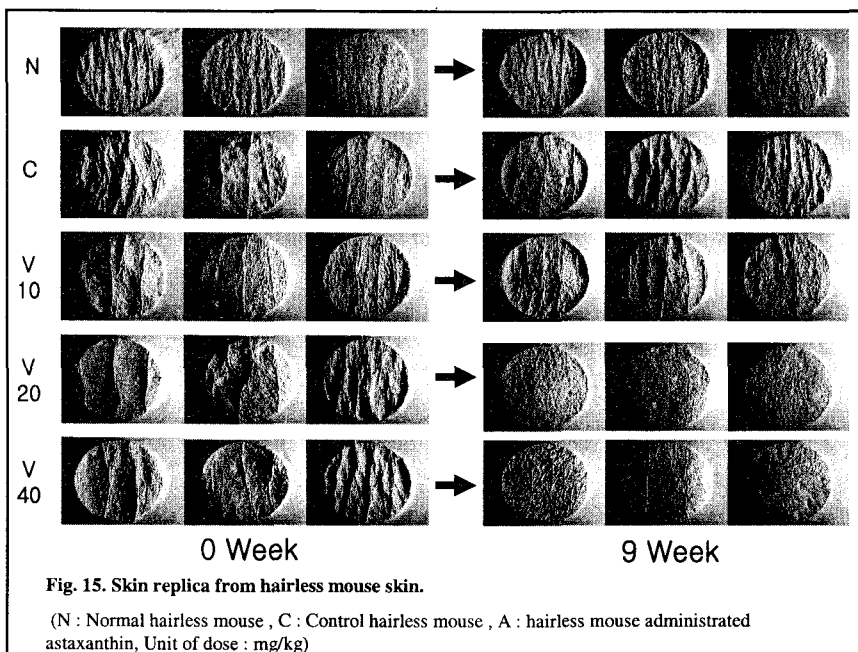
Fig. 12. Interleukin-1 β release from keratinocyte treated with extracts of *Vaccinium uliginosum L.* after UV B irradiation.

(V = Keratinocyte treated with extracts of *Vaccinium uliginosum L.*, UV-C = untreated keratinocyte, Concentration unit of extracts of *Vaccinium uliginosum L.* : mg/mL)

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



**In vivo Anti-wrinkle effects of
*Vaccinium uliginosum L.***



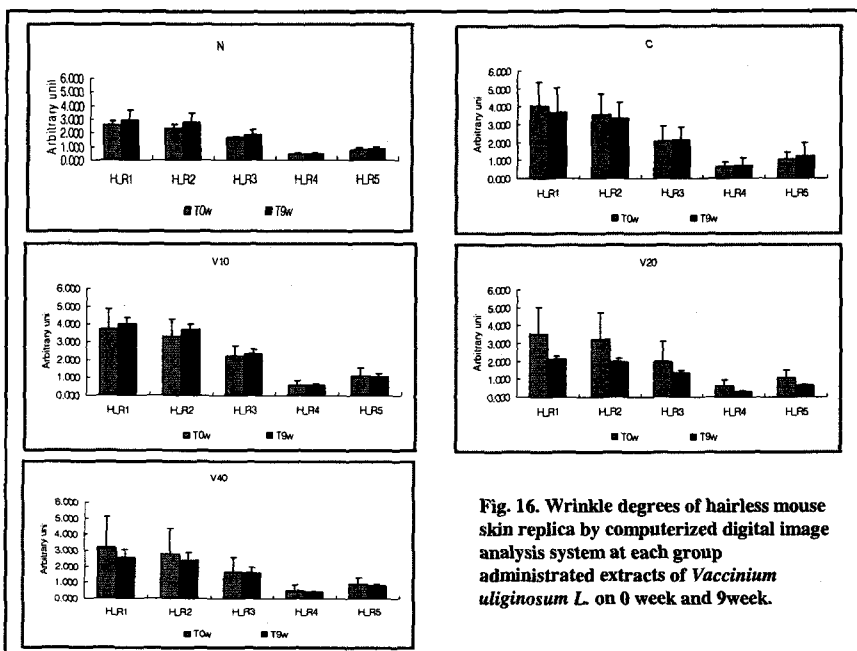


Fig. 16. Wrinkle degrees of hairless mouse skin replica by computerized digital image analysis system at each group administrated extracts of *Vaccinium uliginosum L.* on 0 week and 9week.

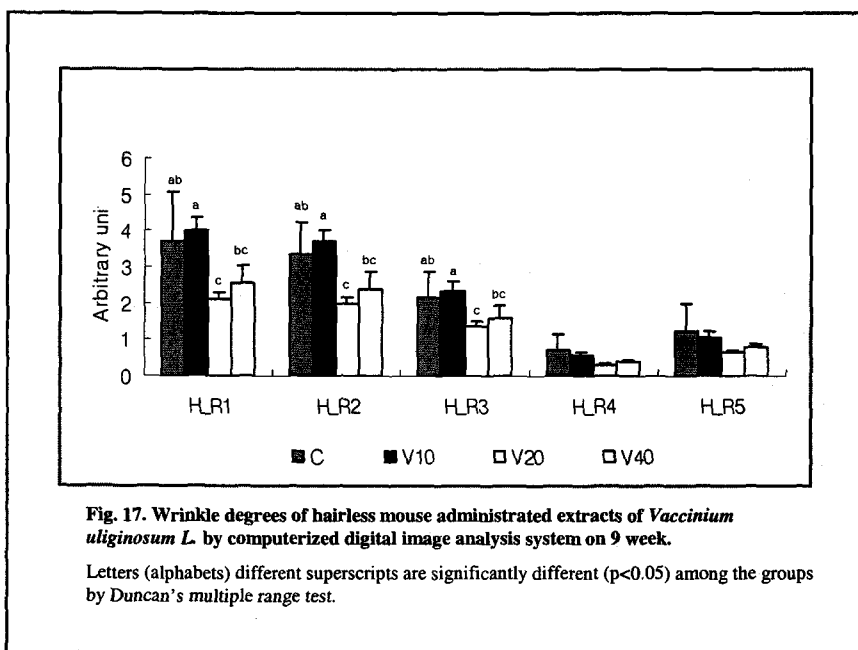
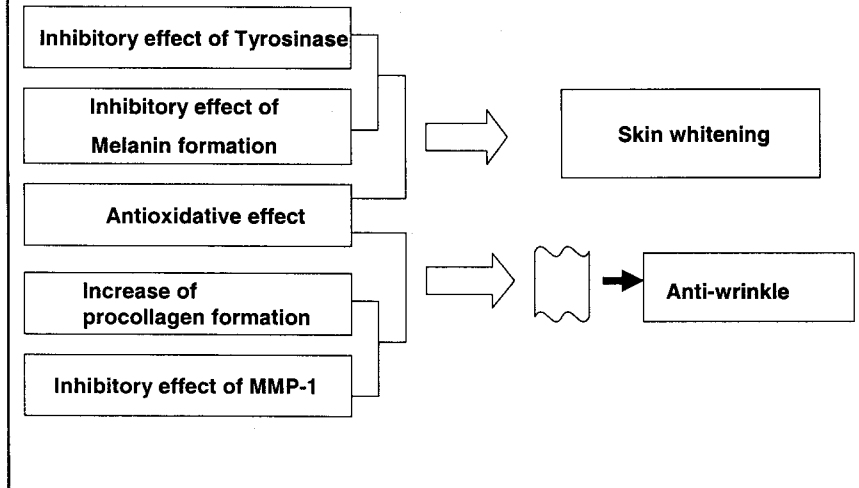


Fig. 17. Wrinkle degrees of hairless mouse administrated extracts of *Vaccinium uliginosum L.* by computerized digital image analysis system on 9 week.

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

Conclusion



Abstract

This study is performed to investigate the effect of water extract from *Vaccinium uliginosum L.*, on melanin production in B 16 melanoma cells, procollagen production and matrix metalloproteinase-1(MMP-1) inhibition in human fibroblast cells.

One hundred grams of the *Vaccinium uliginosum L.* was extracted with 2000 mL of water(90°C, 16h, 2times). The water extracts were lyophilized and stored at 4°C until used. Dry weight yields of extracts of *Vaccinium uliginosum L.* were 3%(w/w).

Extracts from *Vaccinium uliginosum L.* showed scavenger activities on DPPH radical, superoxide anion radical, hydroxyl radical, hydrogen peroxide and singlet oxygen radical. And these substances inhibited release of cytokines from human keratinocyte after UV B exposure.

Therefore we confirmed that extracts from *Vaccinium uliginosum L.* had antioxidative effect.

These substances inhibited purified tyrosinase activity and melanogenesis in B 16 melanoma cells treated/untreated IL-1 α . Moreover this extract stimulated procollagen production and inhibited MMP-1 production in human fibroblast cells treated/untreated IL-1 β . Therefore we confirmed that extracts from *Vaccinium uliginosum L.* had whitening effect.

And these substances decreased degree of wrinkle in hairless mouse skin that induced by UV B irradiation. Therefore we confirmed that extracts from *Vaccinium uliginosum L.* had anti-wrinkle effect.

From the above results, it is possible that *Vaccinium uliginosum L.* may be developed to be an anti-melanogenesis agent and anti-wrinkle agent.