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Master's Thesis of Science in Agriculture

Biochemical Characterization and Use of Mogroside V From *Siraitia grosvenorii*

모그로사이드 V의 생화학적 특성 연구 및 이의 활용방법

August 2020

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Abstract

Mogrosides are cucurbitane-type triterpene glycosides found in certain plants, such as the fruit of the gourd vine, luo han guo (*Siraitia grosvenorii*, monk fruit) that are principle sweet components from fruits. Among them, Mogroside V is the main component composed of 30% extraction yield with over 300-times sweeter compared to sucrose. In addition, it has a low caloric value and low toxicity. However, the price of mogroside V is very high due to lack of efficient purification process for large scale production. In this study, we investigated the purification method of mogroside V by chromatography (HP20 and MPLC). The purity of mogroside V was determined by HPLC and MALDI-TOF. The purified mogroside V was used as solubilizer for idebenone, curcumin, bisdemethoxylcurcumin, oleanolic acid, resveratrol, quercetin, and taxol. The optimization of curcumin for solubilization and its biochemical characterization including antioxidant activities, anti-inflammatory activity, anti-melanin formation were conducted. The anti-inflammation activity on mouse macrophage cell RAW264.7 was studies and water soluble curcumin with mogorisde V effect was lower than that of normal curcumin. The anti-melanin formation such as melanin content assay and celluar tyrosinase activity assay were conducted using B16F10 cells and extra-celluar showed similar performance of water soluble curcumin than curcumin only. In intra-celluar, the effect was higher than normal curcumim. Also cellular tyrosinase showed similar effect than curcumin only.

Keywords: Anti-inflammation, *Siraitia grosvenorii*, Solubilization, Tyrosinase, mogroside V

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Contents

List of Tables

List of Figures

Introduction

1. Mogroside V

Mogroside are cucurbitane-type glycosides found in certain plants, such as the fruit of the gourd vine, luo han guo (*Siraitia grosvenorii*, Monk fruit) [1, 2]. *Siraitia grosvenorii* is known as medicinal and edible plant primarily distributed in Guangxi, China [3]. Among these mogrosides, mogrosides V are measured over 300-times sweeter than sucrose at different concentration levels, low caloric value and low toxicity [4, 5]. Mogroside extracting from monk fruit are generally recognized as safe by the Food and Drug Administration (FDA) [6] and are used as an alternative to sucrose in many countries, such as America, Australia, New Zealand and Japan [7]. In this study mogroside V (MV) efficiently improved the solubility of insoluble drugs it colud be micelle formation (Fig. 1).

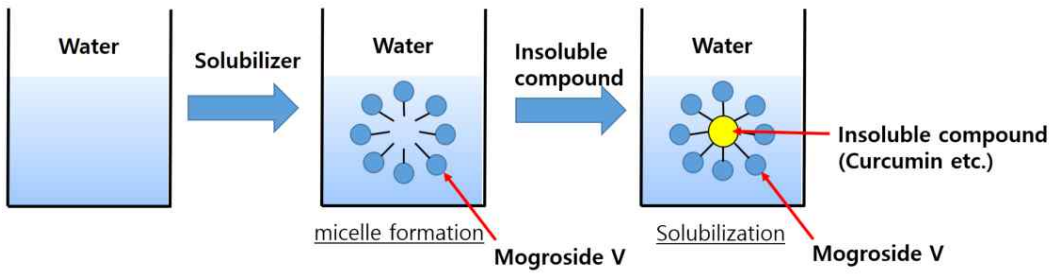


Fig. 1. Micelle illustration of mogorisde V in water

2. RAW264.7 mouse macrophage cell

RAW264.7 cells are macrophages of mouse and have been described as an appropriate model cell line of macrophages because they can perform pinocytosis and phagocytosis [8, 9]. This murine macrophage cell line is frequently used as the model cell for inflammation-related research due to its reproducible response to lipopolysaccharide (LPS) or tumor necrosis factor (TNF)-α [10]. NO, stimulated by LPS, is a typical indicator of inflammatory reactions. In RAW264.7 cells, NO is produced mainly by inducible nitric oxide synthesis (iNOS), which is controlled by heme oxygenase-1 (HO-1), and an anti-inflammatory enzyme. HO-1 is mainly regulated by transcription factor Nrf2, which converts to the nucleus and activates the expression of HO-1 [11]. The macrophage secretes cytokines such as TNF-α and interleukin (IL)-1 or spreads a defense against cytotoxic molecules [12]. RAW264.7 cell line is also used in study to evaluate cell toxicity.

3. B16F10 murine melanoma cell

Melanin is a pigment produced by epidermal melanocytes and is responsible for skin color and for protecting skin from environmental UV damage. In the melanin biosynthesis pathway, tyrosinase catalyzes the rate-limiting steps in which L-tyrosine is hydroxylated to L-3,4-dihydroxyphenylalanine (L-DOPA), and L-DOPA is further oxidized into the corresponding O-quinone. Thus, tyrosinase is a major target in screening inhibitors for melanin synthesis. Several skin depigmenting chemicals such as kojic acid [13] and arbutin [14], which act as tyrosinase inhibitors, have been applied in skin whitening products for the treatment or prevention of abnormal skin pigmentation [15]. It has been reported that microphthalmia-associated transcription factor (MITF), tyrosinase related protein-1 (TRP-1) and tyrosinase related protein-2 (TRP-2) also contribute to the production of melanin [16,17]. Additionally, it has been reported that melanogenesis produces hydrogen peroxide (H_2O_2) and other reactive oxygen species (ROS) that expose the human melanocytes to high levels of oxidative stress [18]. ROS have been shown to play a significant role in the regulation of melanin synthesis, whereas ROS scavengers and inhibitors of ROS generation may down-regulate UV-induced human melanogenesis [19]. The contribution of ROS to melanogenesis has been studied using antioxidants such as N-acetyl cysteine to abolish UVB-induced α -melanocyte stimulating hormone [20]. Stimulation of an endogenous

antioxidant, metallothionein, also suppresses melanogenesis in melanocytes [21]. Furthermore, it is reported that UV light radiation causes the synthesis of ROS in skin [22]. Therefore, the use of antioxidants to protect human skin from the harmful effects of UV radiation is a new trend that has attracted increasing interest in the fields of dermatology and skin care products in recent years [23]. The B16F10 murine melanoma cell line was reported to be a good model for studying human melanoma [24]. Hence, we chose this cell line in the present study to evaluate a natural inhibitor of melanogenesis.

4. Purpose of this study

Mogorisde V (MV) has advantages such as a solubilier, pharmaceutcal agent and natural sweetner in food industry. However, major drawback to industrialization is high price due to lack of purification process effi ciently. To overcome this problem, we investigated efficient refining m ethods for purification of mogroside V by using HP20 and MPLC proc ess. In addition, we performed biocharacterization of purified mogroside V such as cellulr tyrosinase inhibition effect, anti-inflammatory effect, s olubilization ability, chemical stability and cell toxicity in mogroside V. The enhancing of water solububle of these compounds (idebenone, curc umin, bisdemetoxycurcumin, oleanolic acid, resveratrol, quercetin, taxol) with mogroside V were investigated. The water solubilizd compounds a re potential used in food, pharmaceutical and cosmeceutical industries.

Materials and methods

1. Sample preparation

A 20 g of Monk fruit powder were extracted with 100 mL of 60% ethanol in a shaking incubator 40 ℃ for 1 h. Extraction was repeated three times and then the extracts were filtered through Whatman no. 1 filter papre (Piscataway, NJ, USA). The filtrate was concentrated using Heidolph rotary eAvaporator (Heidolph, Schwabach, Germany), concentrated sample was freezed at –80 ℃ and lyophilized at 0 ℃ under 10 Pa for 3 days (EYELA, Tokyo, Japan) and stored at –20 ℃ for further study.

2. Purification of mogroside V using HP-20

Lyophilized Monk fruit sample powder (Mogroside V mixture) added to a final volumn 60% ethanol, followed by centrifugation at 8000 rpm for 3 minutes to precipitate the polymer, and the supernatant was separated. The ethanol present in supernatant was removed under vaccum at 45 ℃ using a rotary evaporator. The concentrated mixture solution was loaded on a 4 × 120 mm open column packed with HP-20 resin. Column was washed by water to remove monosaccharide and disaccharides. Mogroside V was eluted with 10% ethanol. The

eluted solution was concentrated using a rotary evaporator and lyophilized using a freeze dryer.

3. Purification of mogroside V using MPLC

In order to remove the impurity compounds, sample after purifying HP-20 column was applied to amino Reveleris® column (40 μm, 40 g Grace, Los Angeles, USA) on Interchim PuriFlash®430 automated flash chromatography system. The eluent was 10% water at the speed of 15mL/min for 10 min and was eluted with 15% water and 75% acetonitrile for 15 min in order to acquire Mogroside V. The Mogroside V fraction was pooled, acetonitrile was removed using rotary evaporator at 45 ℃, and freeze-dried. Then the purified of mogroside V (10 mg/mL) was confirmed by using MPLC.

4. Thin Layer Chromatography(TLC) analysis

In order to confirm purity, TLC analysis was carried out. Mogroside V obtained above was spotted onto a TLC silica gel plate and developed using a solvent mixture of water: acetonitrile (85:15, v/v). The standard material was 25 mM sucorse, glucose, fructose, 1 mg Mogroside V, and 1 mg of purified Mogroside V. The carbohydrates were visualized by developing the TLC plate in the solution containing 0.5% (w/v) N-(1-napthyl_ ethylenediamine dihydrochloride in methanol and 5% sulfuric acid, following by heating at 125 ℃.

5. Matrix-Assisted Laser Desorption Ionization Time-Of-Flight(MALDI-TOF-MS) analysis

Purified mogroside V was diluted with DMSO-d6 and mixed with 2,5-dihydroxy benzoic acid. The mass spectrum was obtained using a Voyager DE-STR MALDI-TOF mass spectrometer (Applied Biosystems, USA). Mass spctra were obtained in positive reflector mode with a delayed extraction (average of 75 laser shots) method at an acceleration voltage of 25 kV.

6. Analysis of mogroside V using HPLC

Mogroside V was confirmed with analytic HPLC at 210 nm with 2998 photodiode assay detector (Wasters, Massachusetts, USA) using YMC 250 mm × 4.6 mm 5 μm, 12 nm (YMC, Tokyo, Japan) at flow rate 1 mL/min with water and acetonitrile as solvents.

7. Solubilization ability of mogroside V for insoluble compounds

Idebenone, curcumin, bisdemetoxycurcumin, oleanolic acid were purchased from TCI chemical. Resveratrol, quercetin were purchased from Sigma and taxol was purchased from Samyang

biopharmaceuticals. One mg of each insoluble compound (idebenone, curcumin, bisdemetoxycurcumin, oleanolic acid, resveratrol, quercetin, taxol) was mixed with 10 mg of Mogroside V and then 100 μL eathanol was added to the mixture. The mixture solution was put on auto-shaker for 30 min at room temperature and centrifuged at 12,000 rpm for 15 min at 25 ℃. The concentration of each compound in solution was determined using UHPLC-CAD, UV (Ultimate 3000 Charged Aerosol, UV detector, Thermofisher Scientific, Massachusetts, USA and YMC 250 mm × 4.6 mm 5 μm, 12 nm) with standard curves.

8. Optimization of curcumin solubilization conditions with Mogroside V

8.1. Effect of ethanol to curcumin solubility

In order to optimize curcumin solubility curcumin and mogroside V (10:1, w/w) were dissolved in ethanol ranging from 0% to 100%. Mixtures were transfered to 96-well plate and read at 425 nm. The amount of curcumin in the sample was evaluated from a standard curve generated with a curcumin standard curve.

8.2. Effect of mogroside V concentration to solubility

To find the effect of Mogroside V's concentration on curcumin solubility. It was analyzed by setting the range from 10 to 200 mg/mL of mogroside V with 10 mg of curcumin at 80% ethanol. Mixture was transfered to 96 well plate and the concentration of curcumin in each mixture was determined as described above.

8.3. Effect of curcumin concentration

Different curcumin concentration (10-40 mg) was mixed with 7 mg mogroside V at 80% ethanol concentration. The solubilized curcumin concentration was determined as described above.

9. Ferric reducing antioxidant power assay (FRAP)

The antioxidant activity was measured using ferric reducing/antioxidant power (FRAP) assay. Briefly, 0.01 M TPTZ (2, 4, 6-tripyridyl-s-triazine) solubilized in 0.04 M HCl, 0.3 M acetate buffer (pH 3.6) and 0.02 M $FeCl_3.6H_2O$ were thoroughly mixed in a 10:1:1 (v:v:v) ratio just before experiment (FRAP working solution). Then, samples were throughly mixed with FRAP working solution, followed by the addition of distilled water in a 1:30:3 (v:v:v) ratio. Absorbance was measured at 593 nm with a SpectraMax M3 (Mlecular devices Inc., USA). $FeSO_4.7H_2O$ (10-2500 μM) was used as standard. FRAP

value was calculated from stand calibration curve, and the results were expressed as $FeSO_4.7H_2O$ equivalent (mM $Fe2^+$/g Mogroside V)

10. Assay of cell viability

RAW 264.7, a murine monocyte/macrophage cell line and melanoma B16F10 cell were obtained from the Korean Cell Line Bank (Seoul, Korea). Raw 264.7 cells and B16F10 cells were harvested and seeded on 96-well plate (Raw cell: 2.0 × 104 /well and B16F10 cell: 0.5 × 104/well). The cells was growth overnight at 37 ℃, 5% CO_2. Different concentration of samples (0–800 μg/mL) in DMEM was added to each well and the plates were incubated at 37 ℃, 5% CO_2. After 24 h treatment, the cell suspension was removed and cells were washed by PBS buffer. Then cell viability was determined using the EZ-Cytox (Dogen Life science, genetic engineering DAEIL Lab, Seoul, Korea). Water-soluble tetrazolium (WST) and DMEM medium was mixed with ratio 1:9 (v/v), then 100 μL was added to each well. The plates were kept at 37 ℃ for 2 h in the dark and the absorbance at 450 nm was measured using spectrophotometer M3. The cell viability was calculated as follow:

Relative cell viability(%) = (OD_{450} of treated sample/OD of control) x 100

11. Nitric oxide production inhibition assay using RAW264.7 cell

1) Cell culture and treatment

To study the effects of Mogroside V on NO production, Raw 264.7 cells were growth on 96 well-plate (2.0×10^4 /well) with DMEM supplemented with 10% FBS and 1% penicillin and streptomycin under 5% CO2 at 37 ℃. After incubation for 24 h, supernatant was removed and different concentration of sample (1.53 – 200 μg/mL) in DMEM containing 1 μL of lipopolysaccharide was added and incubated at 37 ℃ for 24 h. Cells treated with 1 μL of lipopolysaccharide and 100 μM indomethacin was used as control.

2) NO inhibition measurement

Griess reagent containing 1% (w/v) sulfanilamide in 5% (v/v) phosphoric acid, and 0.1% (w/v) naphthylethlenediamine was prepared. To quantify the nitric oxide released from Raw cells, 80 μL of culture supernantant was mixed with an eual volume of Griess reagent for 20 min. The absorbance was measured at 540 nm using a SPectraMax M3 and the No concentration in the sample was calculated from a sodium nitrite standard curve. The relative NO inhibition was calculated as

follow:

Relative NO inhibition (%) = (1-OD_{540} of sample/OD_{540} control) x 100

12. B16F10 cell melanin synthesis assay

1) Cell culture and treatment

B16F10 cells were cultured in DMEM medium containing 10% heat-inactivated fetal bovine serum (FBS), 1% penicillin and 100 μg/mL streptomycin (Invitrogen, California, USA) at 37 ℃, 5% CO_2. Cells were seeded on 6-well plate (0.5×10^4 cells/well) at 37 ℃, 5% CO_2. After 24 h, different conpcentration of samples (10–200 μg/mL) in DMEM containing 2% FBS, 1% P/S, and 100 nM α-MSH was added to 96-well plate. Cells treated with 100 nM α-MSH in DMEM containing 1% P/S was used as control.

2) Melanin assay

After 72 h treatment, 200 μL of supernantant was taken and put in 96-wel plate. Extra-melanin was measured by at 490 nm using SPectraMax M3. For intra-melanin, cells after 72 h treatment was collected by using 0.05% EDTA-trypsin. Then cells were washed twice by PBS buffer. Then 200 μL 1 M NaOH containing 10% DMSO was added to cells and kept at 80 ℃ for 2 h. The intra-melanin content

was measured as described above.

3) Cellular tyrosinase activity assay

Cells after 72 h treatment were harvested and washed by PBS buffer and centrifuged at 1000 × g for 5 min. Then cells were resuspended in radio immuno precipitation assay buffer (RIPA) for 30 min and centrifuged at 12,000 × g for 20 min. The supernatant was used as crude enzyme solution. 100 μg crude enzyme solution was added in reaction mixture containing 1 mM L-DOPA in 50 mM Na-P buffer pH 6.8 and incubated for 2 h at 37 ℃. The absorbance was measured at 475 nm using SpectraMax M3.

Results and discussion

1. Purification of mogroside V from monk fruit

1.1 Purification mogroside V using HP-20

The monosaccharide and disaccharide in extracted sample were removed by using HP-20. In addition, grosides mixture are in the extract, so it is removed using HP20. The mogrside V obtained through this process is more than 55% purity. This result was verified using the TLC analysis (Fig. 2).

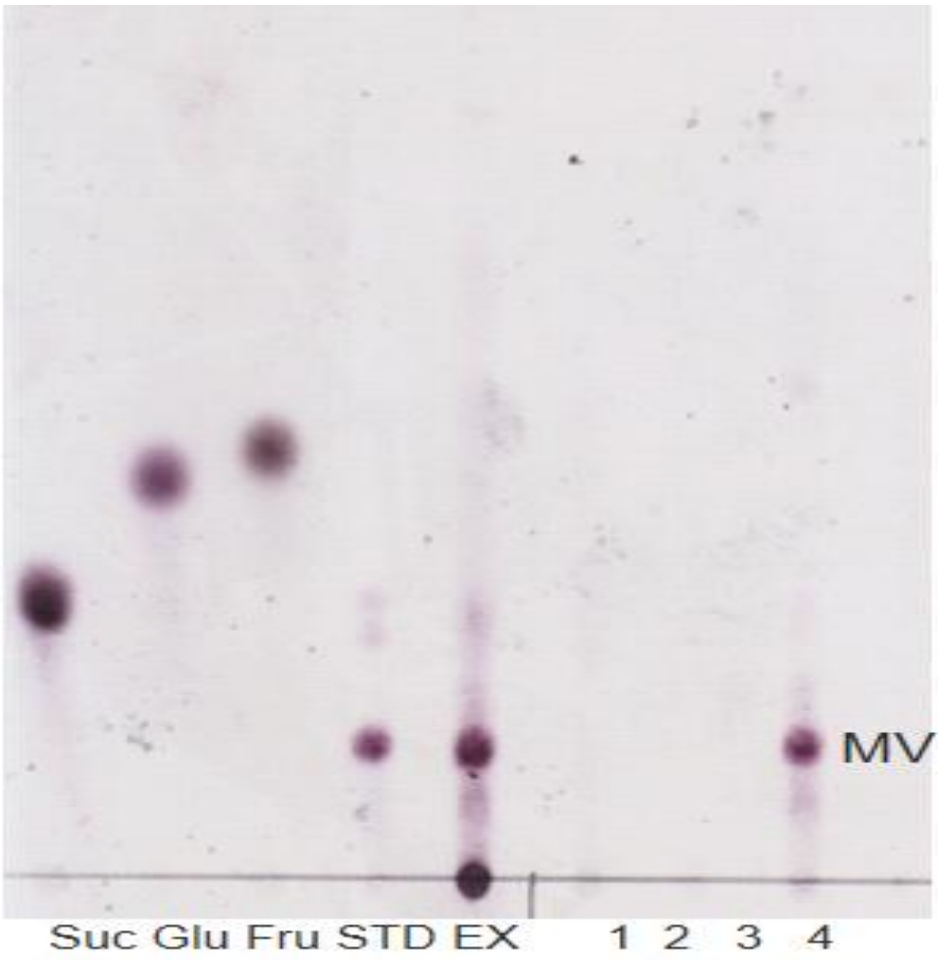


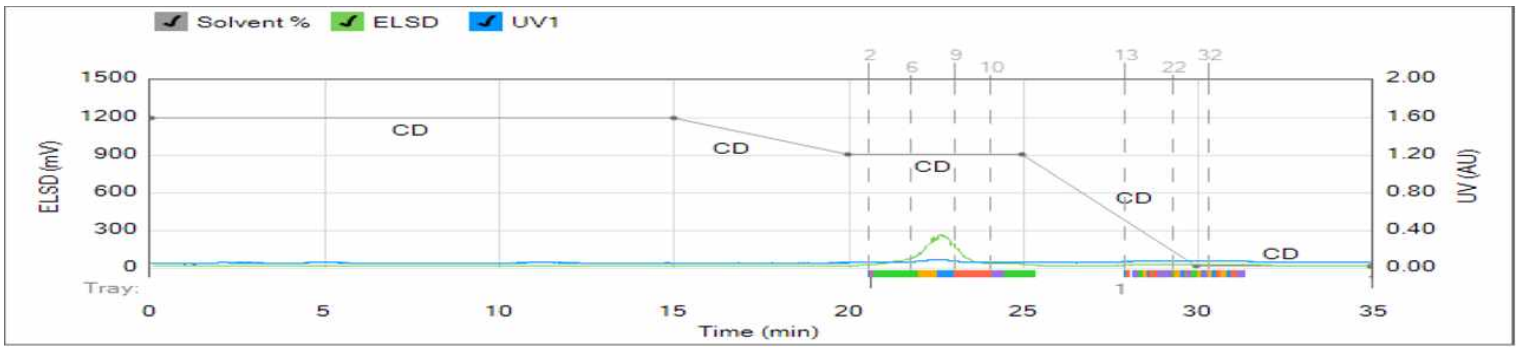
Fig. 2. Thin layer chromatography of mogroside V sample after removal of saccharides using HP-20

Suc: 20 mM Sucrose; Glu: 20 mM glucose; Fru: 20 mM fructose; STD: mogroside V; EX: monk fruit extracted; Lane 1: 10% ethanol elution; Lane 2: 30% ethanol eliution; Lane 3: 50% ethanol elution; Lane 4: 70% ethanol elution. TLC was done on a silica gel 60 by one ascent with the solvent of water : acetonitrile [85:15 (v/v)].

1.2 Purification mogroside V using MPLC

HP-20 treated sample was purified using Interchim PuriFlash®430 automated flash chromatography system with an Amino 40 μm 40 g Reveleris® column (Grace, Los Angeles, USA). The mogroside V fraction was pooled, removed of their acetonitrile using rotary evaporator at 45 ℃, and freeze-dried. Dissolved in distilled water with 10 mg/mL. We proceeded to obtain high purity mogroside V through MPLC, and mogroside V obtained through the MPLC process was analyzed using HPLC. The results are shown in (Fig. 3, 4).

(A)



(B)

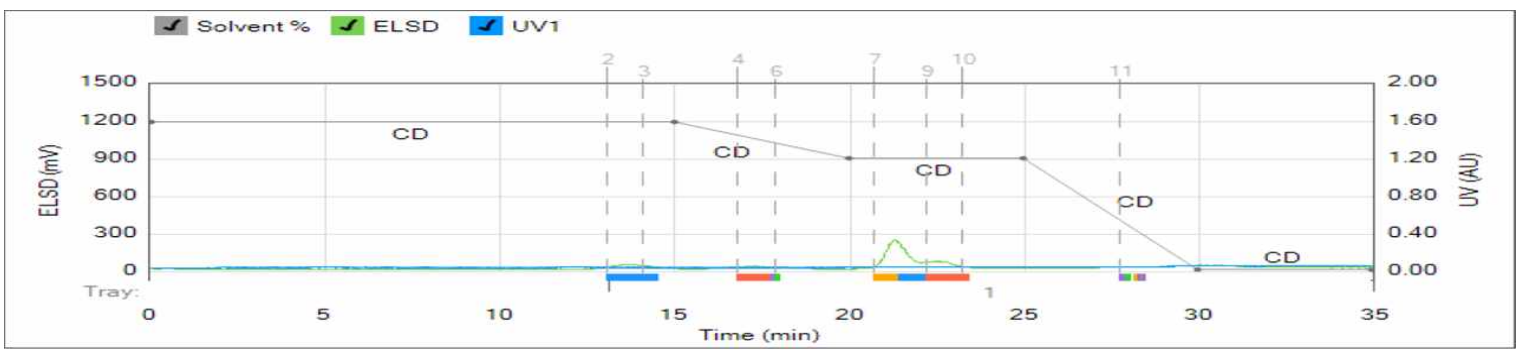


Fig. 3. MPLC chromatogram of mogroside V (A) purified mogroside V (B) HP-20 treated mogrosides sample

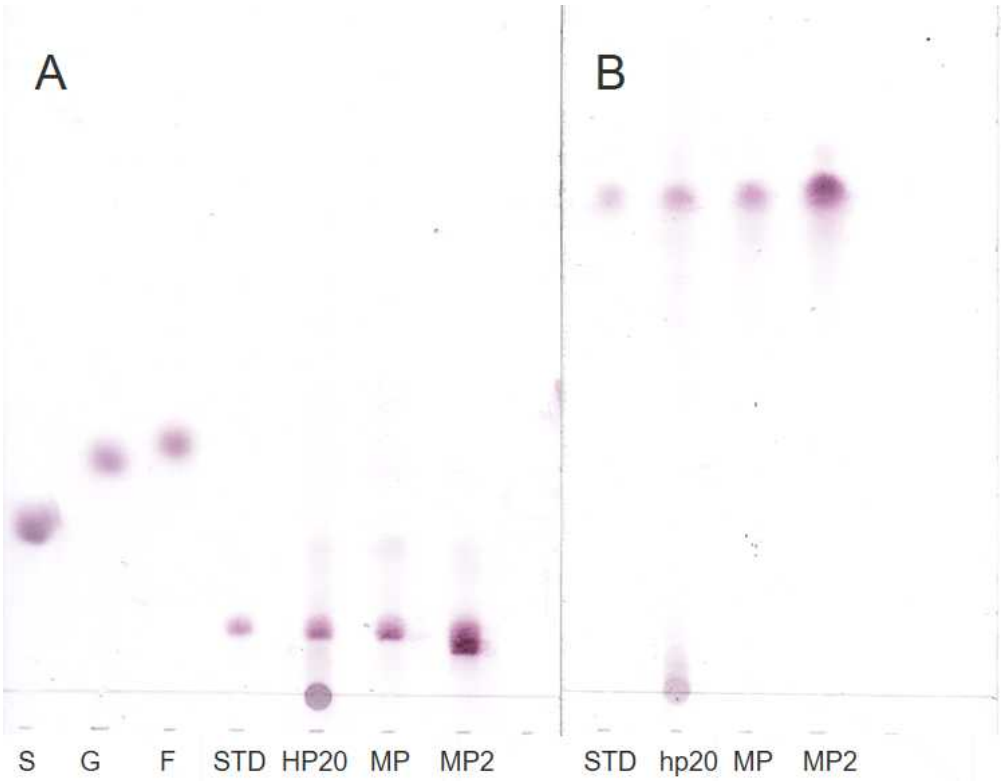


Fig. 4. Thin layer chromatography of mogroside V partially purified using MPLC

Suc: 20 mM Sucrose; Glu: 20 mM glucose; Fru: 20 mM fructose;

STD: mogroside V; HP20: Saccharides were removed Mogrosides; MP: purified mogroside V; MP2: concentrated MP sample. TLC was done on a silica gel 60 by one ascent with the solvent of A: water : acetonitrile [85:15 (v/v)], B: nitromethane : 1-propanol : water [2:5:1.5 (v/v/v)]

2. Analysis of mogroside V using HPLC and MALDI-TOF

2.1 Analysis of mogroside V using HPLC

The results of saccharide (sucrose, glucose, fructose, olligosaccharides or impurity compounds were removed and the mogroside V was analyzed by HPLC-PDA at 210 nm, YMC 250 mm × 4.6 mm 5 μm, 12 nm (YMC, Japan) at flow rate 1 mL/min with water and acetonitrile. The purity of mogroside V obtained through MPLC was confirmed, the purity was more than 92%. The results are shown in (Fig. 5).

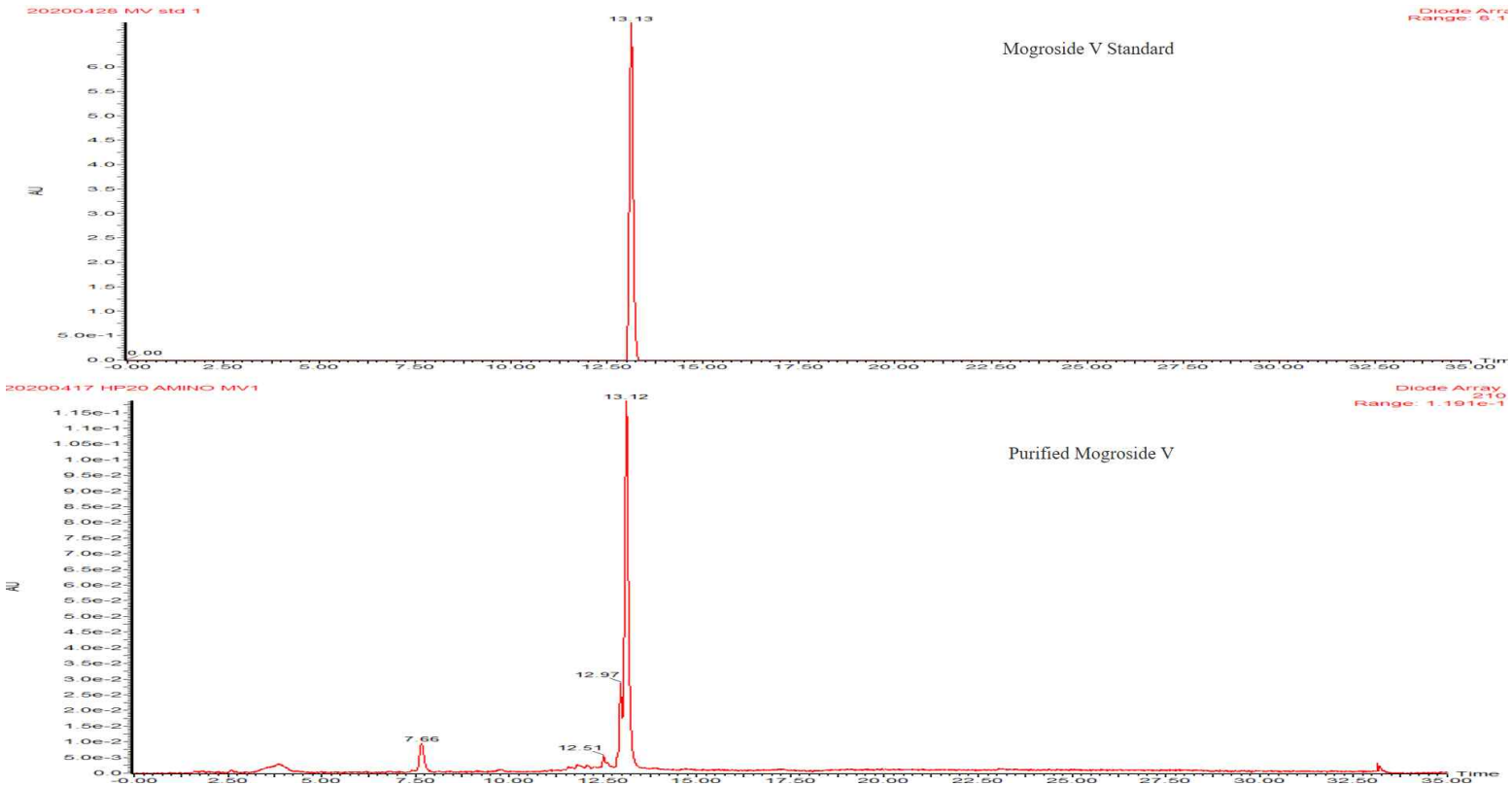


Fig. 5. HPLC chromatogram of mogroside V standard and purified sample

2.2 Molecular weight measurement of Mogroside V

MALDI-TOF-MS analysis was used to measure molecular weight and purity of Mogorisde V (Fig. 6**)**. Molecular weight of standard mogroside V was 1287.4 g/mol and purified mogorisde V were 1309.9 g/mol and 1325.9 g/mol. A sodium ion and potassium ion were combined during the analysis. Molecular weight of a sodium ion is 22.99 g/mol and potassium ion is 39.09 g/mol. Given that there are not many matrices on the analysis graph, the purity was also sufficient.

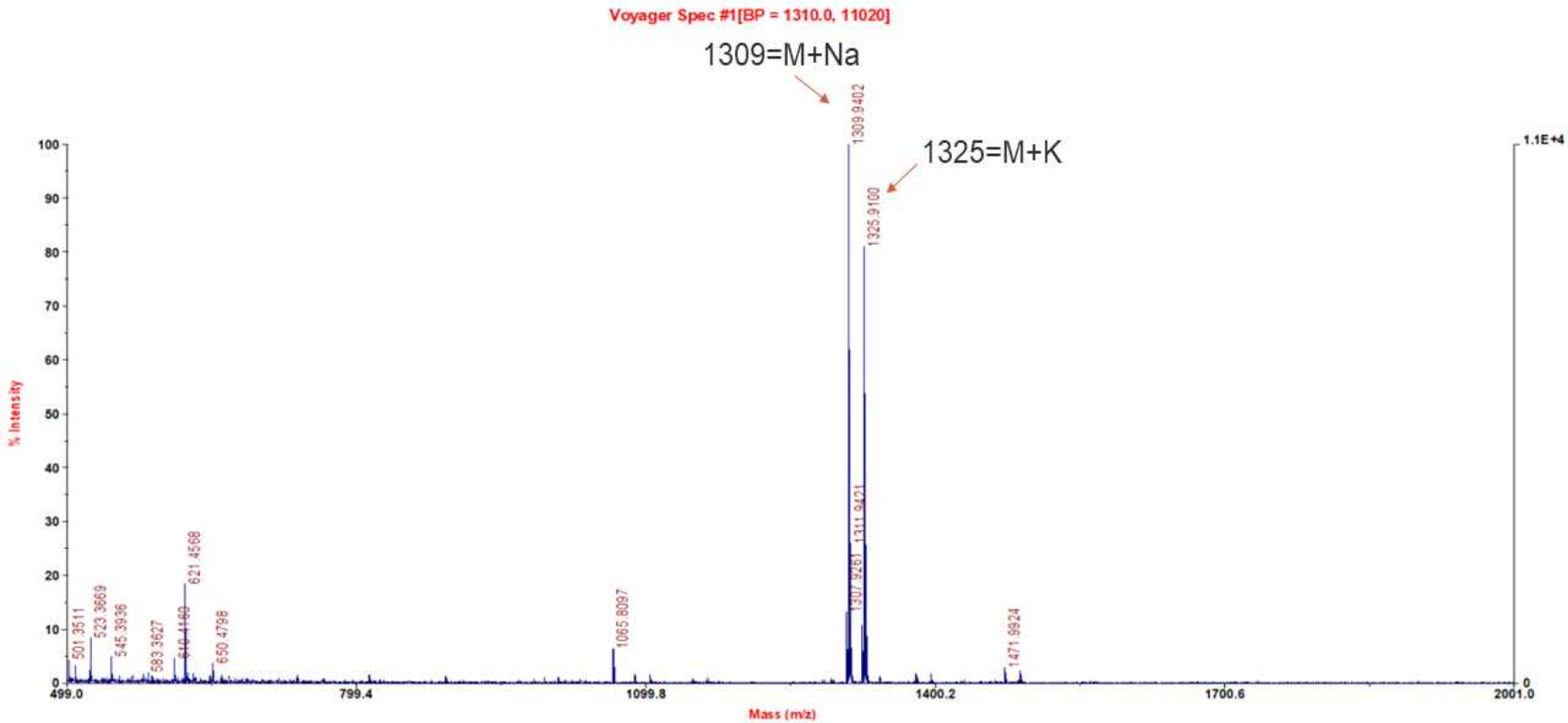


Fig. 6. MALDI-TOF-MS spectra of Mogorisde V

3. Solubilization insoluble compounds with Mogroside V

3.1 Idebenone

Idebenone [25] is an organic compound of the quinone family. It is known that idebenone has a good effect on brain-related disease [26, 27], as well as a decrease in cardiovascular disease [28]. Recently, the cosmetic industry is paying attention to idebenone, but it having difficulty in using it due to poor water solubility [29].

The results of the solubilization test using mogroside V confirmed by UHPL-CAD/UV detector (Fig. 7) that the water-solubility increase 78 times (78.0 μg/ml) compared to Idebenone only (Table 1). This data shows the potential of moroside V as a natural solubilizer.

3.2 Resveratrol

Resveratrol (3,4',5 trihydoxystilbene) is a phytoalexin and a member of the stilbene family, which is commonly found in some spermatophytes. It is produced in plants in response to stress, injury, infection or UV radiations. The food sources of this flavonoid are grapes, peanuts, wine, blueberries, bilberries, dark chocolate and tea. The health benefits of resveratrol were first highlighted in the early 1990s, when it was noticed that in spite of the consumption of high fat diet the French had low incidence of coronary heart diseases [30]. These potential therapeutic and prophylactic applications are limited by the low bioavailability caused by its physical properties. Additionally, resveratrol has low water solubility and stability making its clinical success a formidable technological and medical challenge [31]. The results of the solubilization test using mogroside V confirmed by UHPLC (Fig. 8) that the water-solubility increase 88.4 times (88.4 μg/ml) compared to resveratorl only (Table 1) this work is to present results of improvement of solubility of resveratrol through mogroside V.

3.3 Oleanolic acid

The natural product Oleanolic acid (3β-hydroxyolean-12-en-28-oic acid) naturally derived triterpene, is found in many traditional Chinese medicines. Its many pharmacological activities include hepatoprotective, anti-tumour, antibacterial, anti-inflammatory and antiulcer effects. but its poor solubility often leads to poor bioavailability [32].

The results of the solubilization test using mogroside V confirmed by UHPL-CAD/UV detector (Fig. 9) that the water-solubility increase 23.9 times (23.9 μg/ml) compared to oleanolic acid only (Table 1). This data shows the potential of mogrosdie V.

Table 1. The UHPLC quantification of water solube idebenone, resveratrol and oleanolic acid

Solubilization	Compounds		
	Water	Mogorisde V	Relative solubility
	μg/ml	μg/ml	(fold)
Idebenone concentration	0.000 ± 0.00	78.0 ± 0.3*	78
Resveratrol concentration	0.000 ± 0.00	88.4 ± 0.6*	88.4
Oleanolic acid concentration	0.000 ± 0.00	23.9 ± 0.6*	23.9

* Significant difference ($p<0.01$)

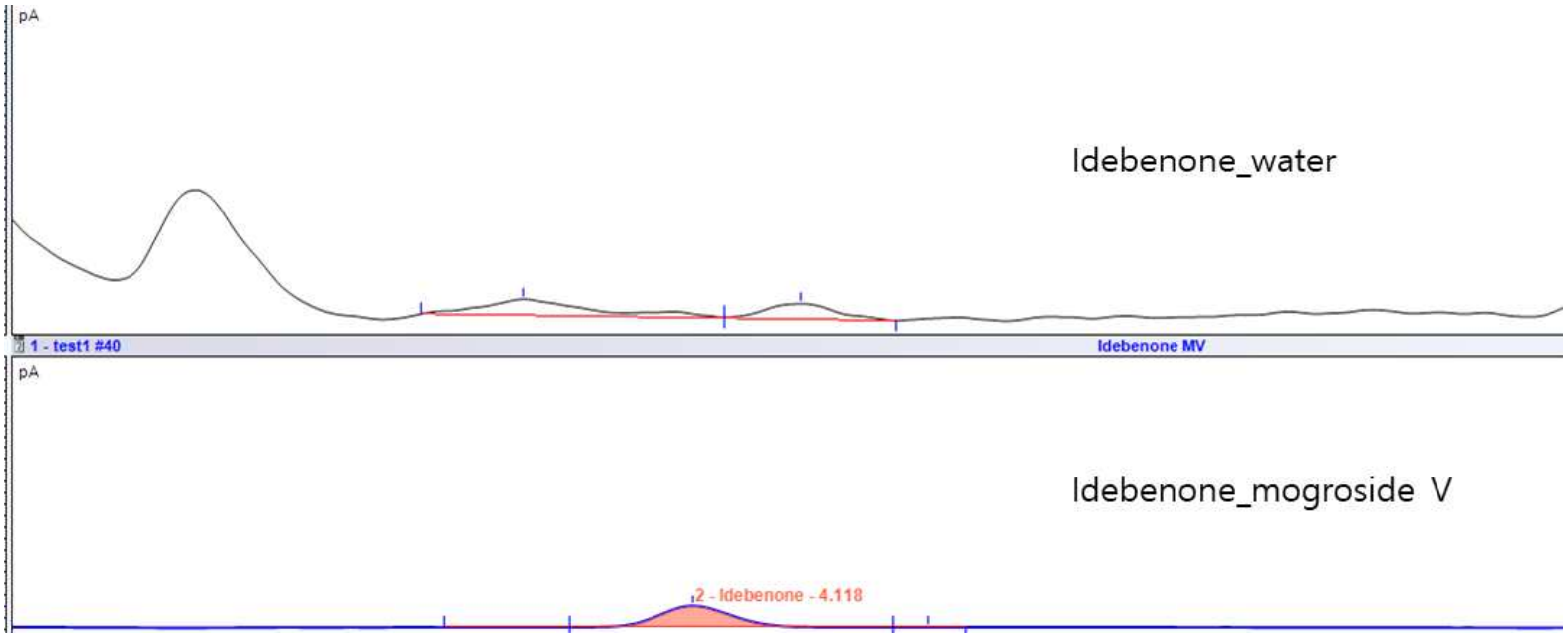


Fig. 7. The UHPLC chromatogram of water soluble idebenone

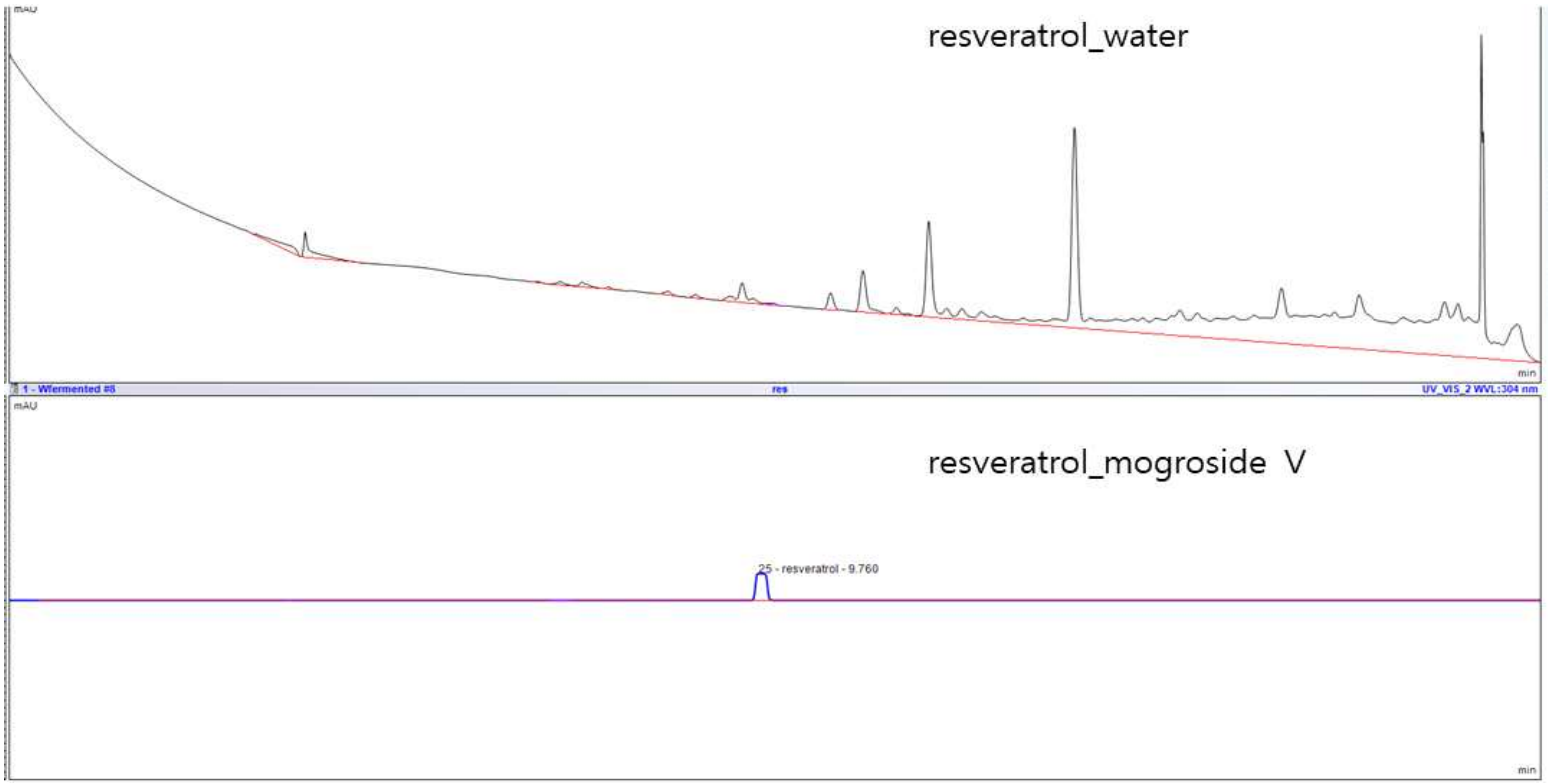


Fig. 8. The UHPLC chromatogram of water soluble resveratrol

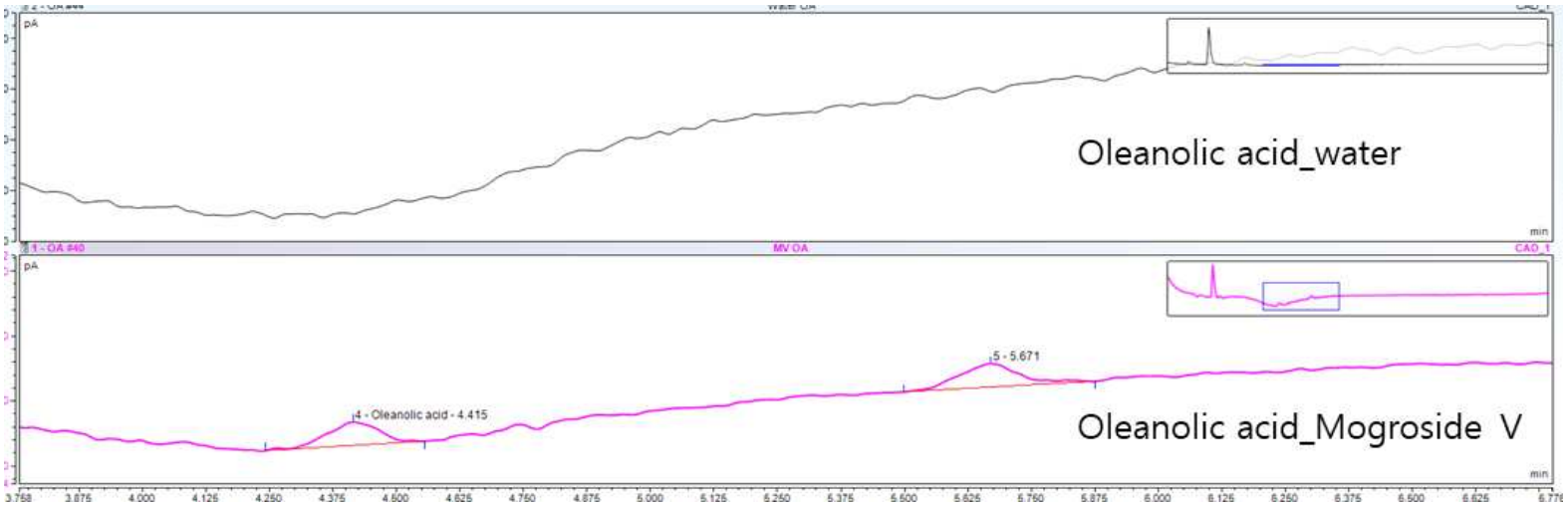


Fig. 9. The UHPLC chromatogram of water soluble oleanolic acid

3.4 Curcumin

Curcumin [33] is a polyphenols that is mainly present in tumeric (*Curcuma longa* L). Curcumin is not only known to have an excellent effect in anti-oxidant [34, 35], it is also known to have anti-cancer effect [36] and good effect related to diabetes [37]. Still, it has water-insolubility, making it difficult to be used as a food material for which organic solvents cannot be used.

The results of the solubilization test using mogroside V confirmed by UHPLC (Fig. 10) that the water-solubility increased 230 times (230.0 μg/ml) compared to curcumin only (0.0 μg/ml) (Table 2).

3.5 Bisdemethoxycurcumin

The most important chemical components of turmeric are a group of c ompounds called curcuminoids, which include curcumin, demethoxycurc umin, and bisdemethoxycurcumin. Bisdemethoxycurcumin is used as a pigment and Nutraceutical with antimutagenic properties [38, 39]. All t hree of the curcuminoids found in Curcuma longa have been shown to have antioxidant properties, However bisdemethoxycurcumin is more res istant than the others to alkaline degradation [40]. The results of the s olubilization test using mogroside V confirmed by UHPLC (Fig. 11) th at the water-solubility increased 24.0 times (24.0 μg/ml) compared to water-dissoled bisdemethoxycurcumin (0.0 μg/ml) (Table 2).

Table 2. The UHPLC quantification of water soluble curcumin and bisdemethoxycurcumin

Solubilization	Compounds		
	Water	Mogorisde V	Relative solubility
	μg/ml	μg/ml	(fold)
Curcumin concentration	0.000 ± 0.00	230.0 ± 0.21	230
Bisdemethoxy curcumin concentration	0.000 ± 0.00	24.0 ± 0.1*	24

* Significant difference ($p<0.01$)

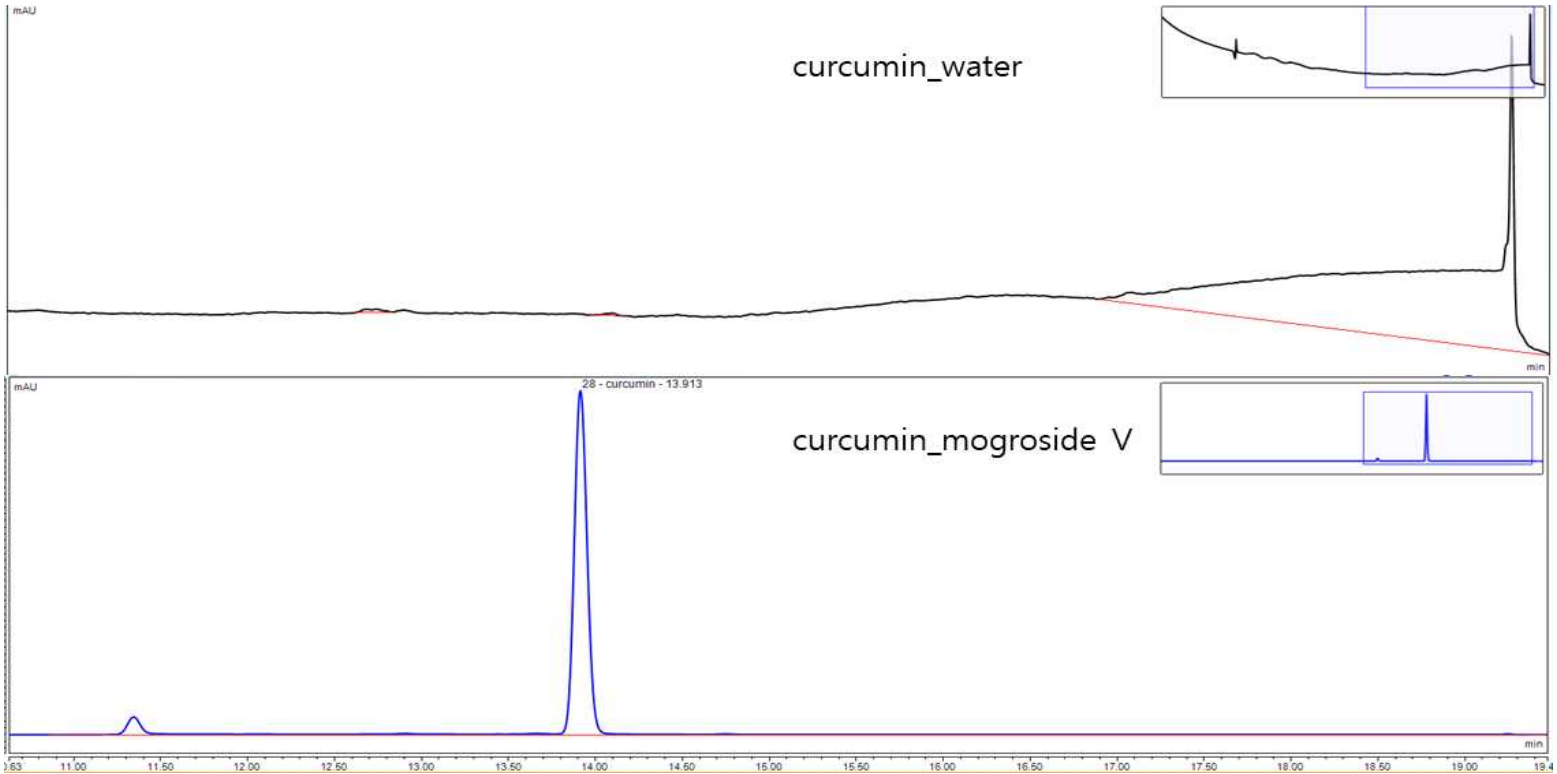


Fig. 10. The UHPLC chromatogram of water soluble curcumin

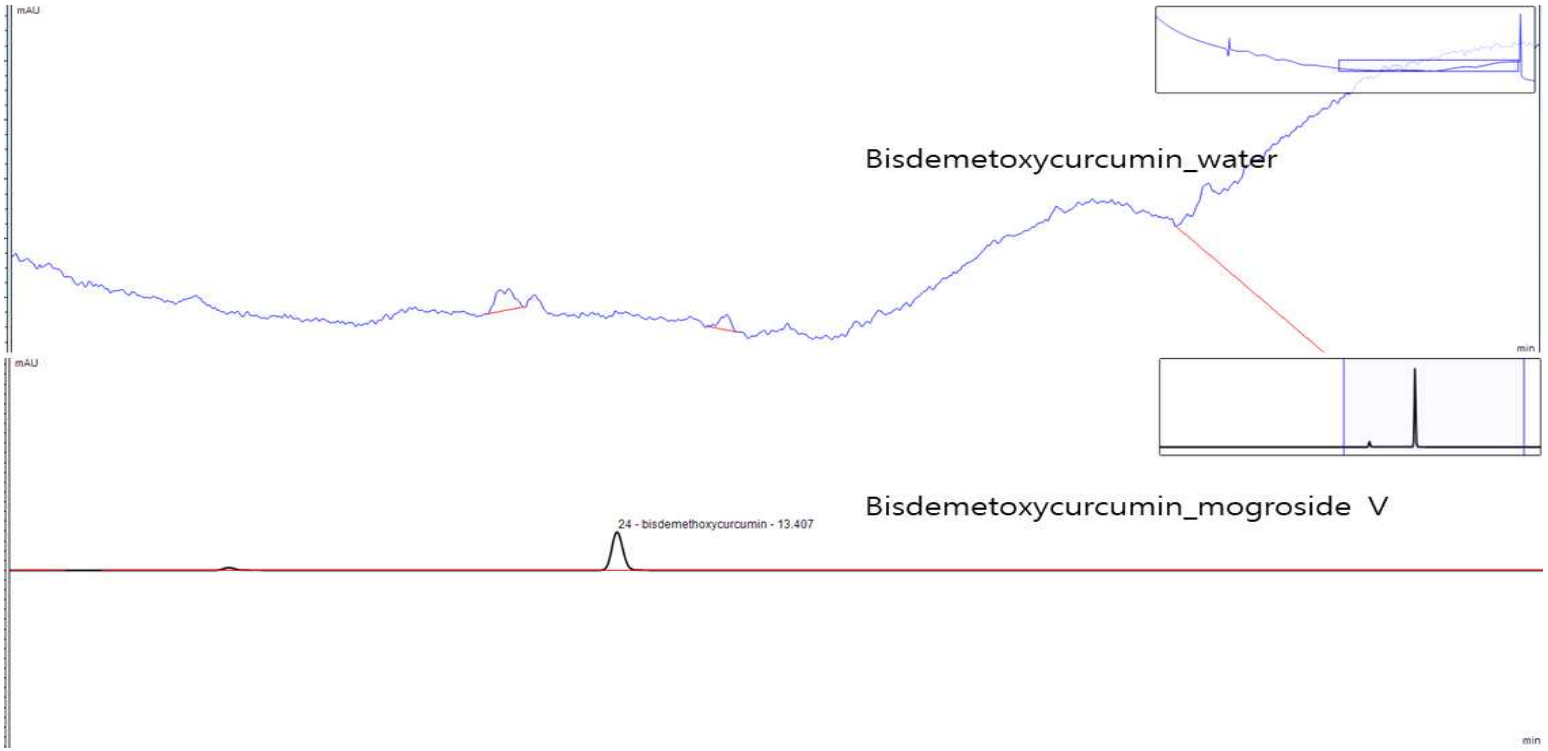


Fig. 11. The UHPLC chromatogram of water soluble bisdemetoxycurcumin

3.6 Taxol

Paclitaxel (Taxol) is a chemotherapic drug specifically effective against prostate, ovarian, breast, and lung cancer. Its primary mechanism of action is related to the ability to stabilize the microtubules and to disrupt their dynamic equilibrium [41, 42, 43, 44]. Taxol inhibits cell proliferation by promoting the stabilization of microtubules at the G-M phase of the cell cycle, by which depolymerization of microtubules to soluble tubulin is blocked [45, 46, 47]. Taxol was originally isolated from the bark of the Pacific yew, *Taxus brevifolia* [48]. The limited availability of mature yew trees, slow growth rate of cultivated plants, and the low yield of the taxol has resulted in its high cost and also has raised concerns about environmental damage from excessive exploitation of wild trees [49]. Still, it has low water-insolubility, making it difficult to be used as a drug or food material for which organic solvents cannot be used. The results of the solubilization test using mogroside V confirmed by UHPLC (Fig. 12) that the water-solubility increased 165.8 times (39.8 μg/ml) compared to taxol in water (0.24 μg/ml) (Table 3).

3.7 Quercetin

Quercetin, a flavonoid found in fruits and vegetables, has unique biological properties that may improve mental/physical performance and reduce infection risk [50]. These properties form the basis for potential benefits to overall health and disease resistance, including anti-carcinogenic, anti-inflammatory, antiviral, antioxidant, and psychostimulant activities, as well as the ability to inhibit lipid peroxidation, platelet aggregation and capillary permeability, and to stimulate mitochondrial biogenesis [51]. Despite these advatages, low water solubility is limited in itrs use as a food material. In order to increase the bioavailability and usability of quercetinm, this work conducted using natural solubilizer such as mogroside V. The results of the solubilization test using mogroside V confirmed by UHPLC (Fig. 13) that the water-solubility increased 131 times (10.5 μg/ml) compared to quercetin only (0.08 μg/ml) (Table 3).

Table 3. The UHPLC quantification of water solube taxol and quercetin

Solubilization	Compounds		
	Water	Mogorisde V	Relative solubility
	μg/ml	μg/ml	(fold)
Curcumin concentration	0.24 ± 0.00	39.8 ± 0.13*	165.8
Bisdemethoxy curcumin concentration	0.08 ± 0.00	10.5 ± 0.14*	131.6

* Significant difference ($p<0.01$)

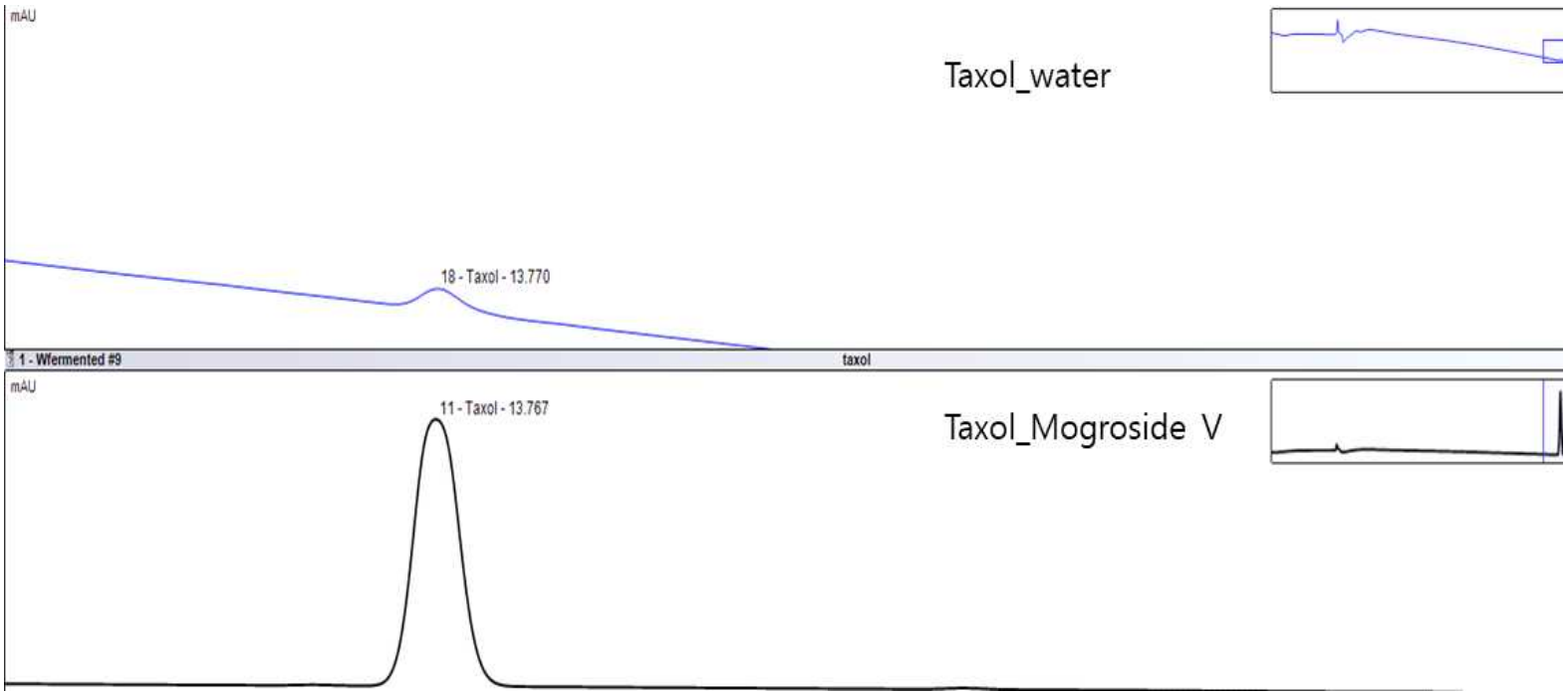


Fig. 12. The UHPLC chromatogram of water soluble taxol

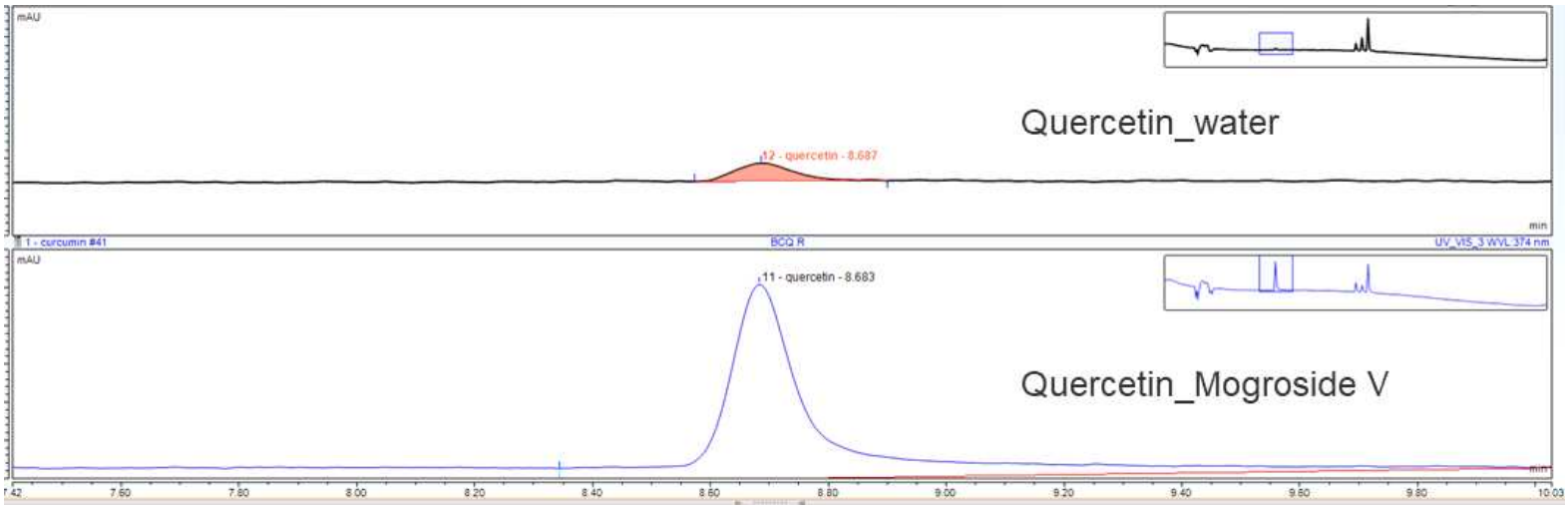


Fig. 13. The UHPLC chromatogram of water soluble quercetin

4. Curcumin solubilization conditions with mogroside V for improved water solubility

The benefits of curcumin are described in section 3.4 of this paper. In addition, when analyzing various insoluble compounds using mogroside V, the most efficient was curcumin. Based on these advantages, optimization conditions to create curcumin-mogroside V complex were conducted and divided into three conditions.

First Effect of ethanol to curcumin solubility. In Effect of ethanol to curcumin solubility, when curcumin was dissolved at 0% to 100% ethanol concentration, it showed the best efficiency at 80% (Table 4).

Second In the effect of mogroside V concentration to solubility, the efficiency of dissolving mogroside V from 0 mg to 200 mg of 80% ethanol and adding 10 mg of curcumin was confirmed. At this time, the highest efficiency was found at 75 mg/10 mg (Table 5).

Third In effect of curcumin concentration to solubility, for the highest solubilized curcumin value was confirmed when 75 mg mogrosidse V was dissolved in 80% ethanol and the curcumin concentration was changed from 1 mg to 40 mg, and 75 mg mogroside V / curcumin 10 mg was identified as the most optimal condition (Table 6). And the optimized curcumin-mogroside V complex was analyzed by HPLC (Fig 14).

Ethanol concentration (%)	0	10	20	30	40	50	60	70	**80**	90	100
Curcumin concentration (mg/ml)	0.07 ± 0.01	0.09 ± 0.01	0.35 ± 0.01	1.32 ± 0.04	2.12 ± 0.07	2.50 ± 0.06	3.72 ± 0.09	4.41 ± 0.05	**6.78 ± 0.13**	6.74 ± 0.05	6.88 ± 0.26

Table 4. Effect of ethanol to curcumin solubility

mogroside V concentration in 80% ethanol (mg/ml)	10	30	50	**75**	100	125	150	200
Curcumin concentration (mg/ml)	0.66 ± 0.03	2.44 ± 0.12	5.61 ± 0.04	**6.18 ± 0.06**	6.42 ± 0.15	6.34 ± 0.06	6.38 ± 0.15	6.40 ± 0.03

Table 5. Effect of mogroside V concentration to curcumin solubility

Curcumin concentration in 80% ethanol with 75 mg/mL mogroisde V (mg/ml)	1	3	5	7	**10**	12	15	17	20	25	30	40
Amount of curcumin (mg/ml)	0.8 ± 0.01	2.74 ± 0.10	4.74 ± 0.20	6.26 ± 0.09	**6.70* ± 0.00**	6.63* ± 0.05	6.52* ± 0.00	6.79* ± 0.06	6.48 ± 0.07	6.63 ± 0.17	6.52* ± 0.06	6.85* ± 0.04

* Very significant ($p<0.001$)

Table 6. Effect of curcumin concentration to curcumin solubility

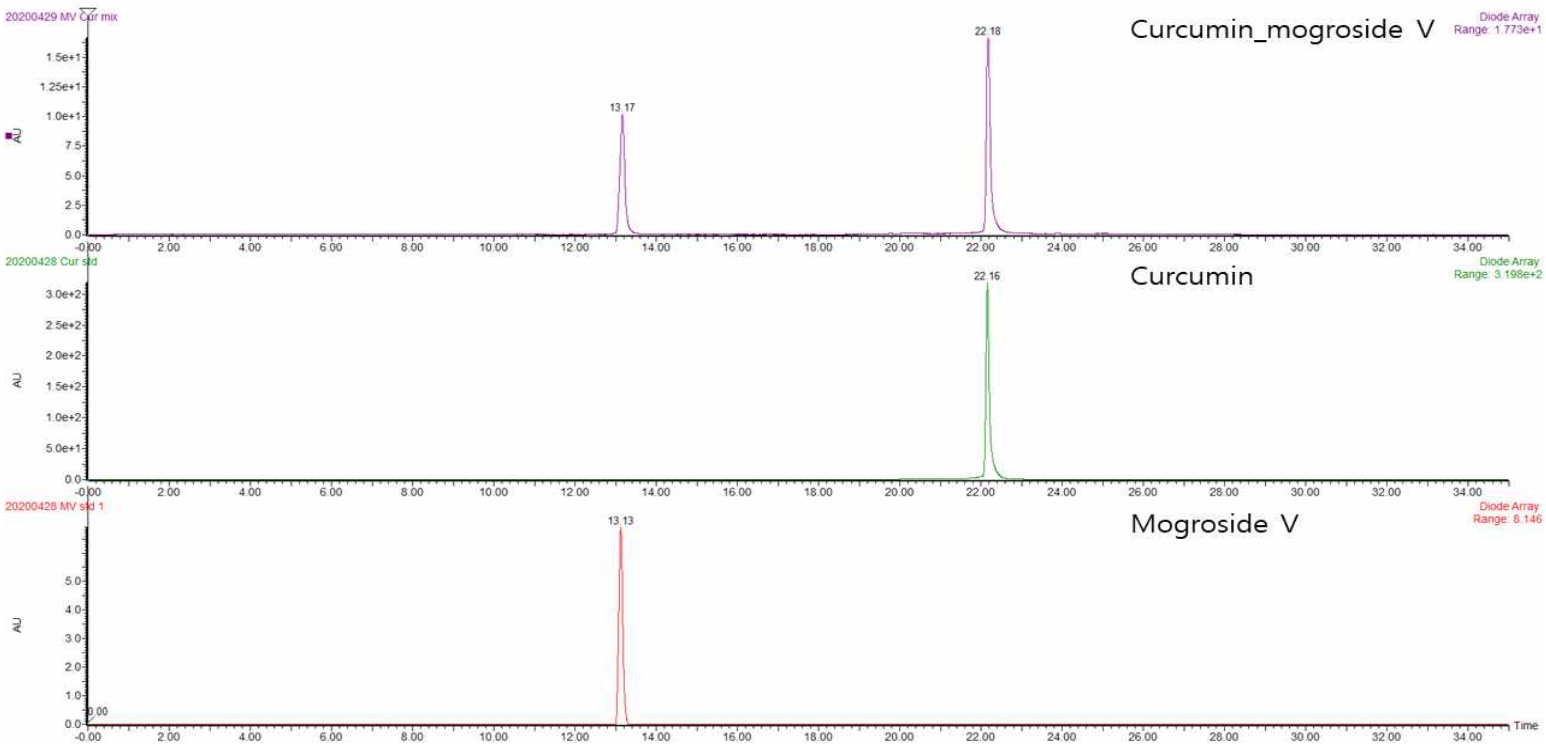


Fig. 14. The HPLC chromatogram of water soluble curcumin

5. Analysis of antioxidant activity

In this study, the antioxidant activities of mogroside V, curcurmin, curcumin-mogroside V were studied using FRAP assay, In general, the antioxidant activity of ethanol dissolved curcumin sample had higher than water dissolved sample approximately 3.9 times. 10 μg curcumin in ethanol sample was analyzed as a curcumin final concentration. Accordingly, curcumin-mogroside V complex sample was prepared for concentration, in this case the mogroside V concentration was 750 μg. To check the antioxidant effect of mogroside V in the water solul curcumuin, same concentration of mogroside V was also analyzed. This data shows antioxidant effect of curcumin in water sample is about 4 times lower than curcumin in ethanol. However, when curcumin, which was not soluble in water at all, when dissolved using mogroside V, the antioxidant effect was weak, but it was confirmed to exist.

Probably the molecules of the mogroside V present in the area increased the viscosity therefore decreasing the mobility and also competing for the interactions with molecules of curcumin, which reduced the interaction between curcumin and Fe^{3+} molecules.

Sample	FRAP (μmol Fe (II))
Mogroside V***	46.66 ± 2.34
Curcumin*	372.44 ± 7.09
Curcumin** with mogroside V	95.88 ± 4.84

Table 7. Determination of FARAP for mogroside V, curcumin, curcumin with mogroside V. All samples are expressed as means ± standard deviation of triplicate analysis. FRAP; Ferric reducing antioxidant power assay. To make the final concentration the same, it was based on curcumin dissolved in ethanol (*: 10 μg curcumin in ethanol, **: 10 μg curcumin with 750 μg of mogroside V, ***: only 750 μg of mogroside V to see the effect of mogroside V in curcumin). Fe(II): $FeSO_4$ equivalent.

6 Cell cytotoxicity of mogroside V

To evaluate cytotoxicity, survival rates were measured by treatment the compound (Cur, MV, Cur-MV) in RAW264.7 cells (Fig. 15) and B16F10 melanoma cells (Fig. 16). We LC50 value obtained at the both cell line for further study. Test have shown mogroside V does not show cell toxicity at the RAW264.7 and B16F10 cells up to 1600 μg/mL. In RAW264.7 cell viability data, normal curcumin LC50 value was 25.3 μg/mL water soluble curcumin was 28.1 μg/mL. When comparing LC50 values, it was confirmed that water soluble curcumin was less toxic than normal curcumin. In B16F10 case, normal curcumin LC50 value was 79.2 μg/mL and water soluble curcumin LC50 value was 66.1 μg/mL. the toxicity of water soluble curcumin was higher than that of normal curcumin.

However, these toxic values were set for sample concentrations in future experiments, so they are not mean good or bad effect.

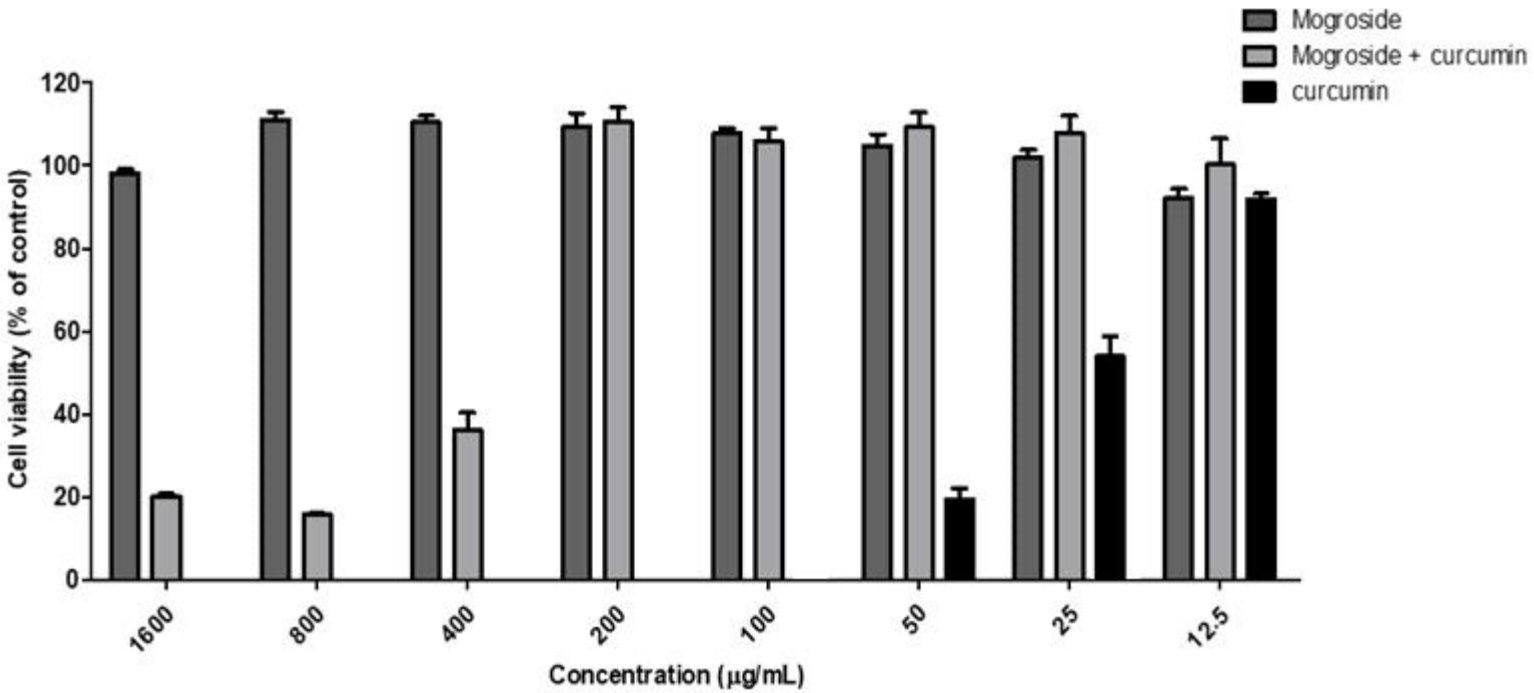


Fig. 15. Effect of mogroside V, curcumin, water soluble curcumin with mogroside V in cultured RAW264.7 cells.

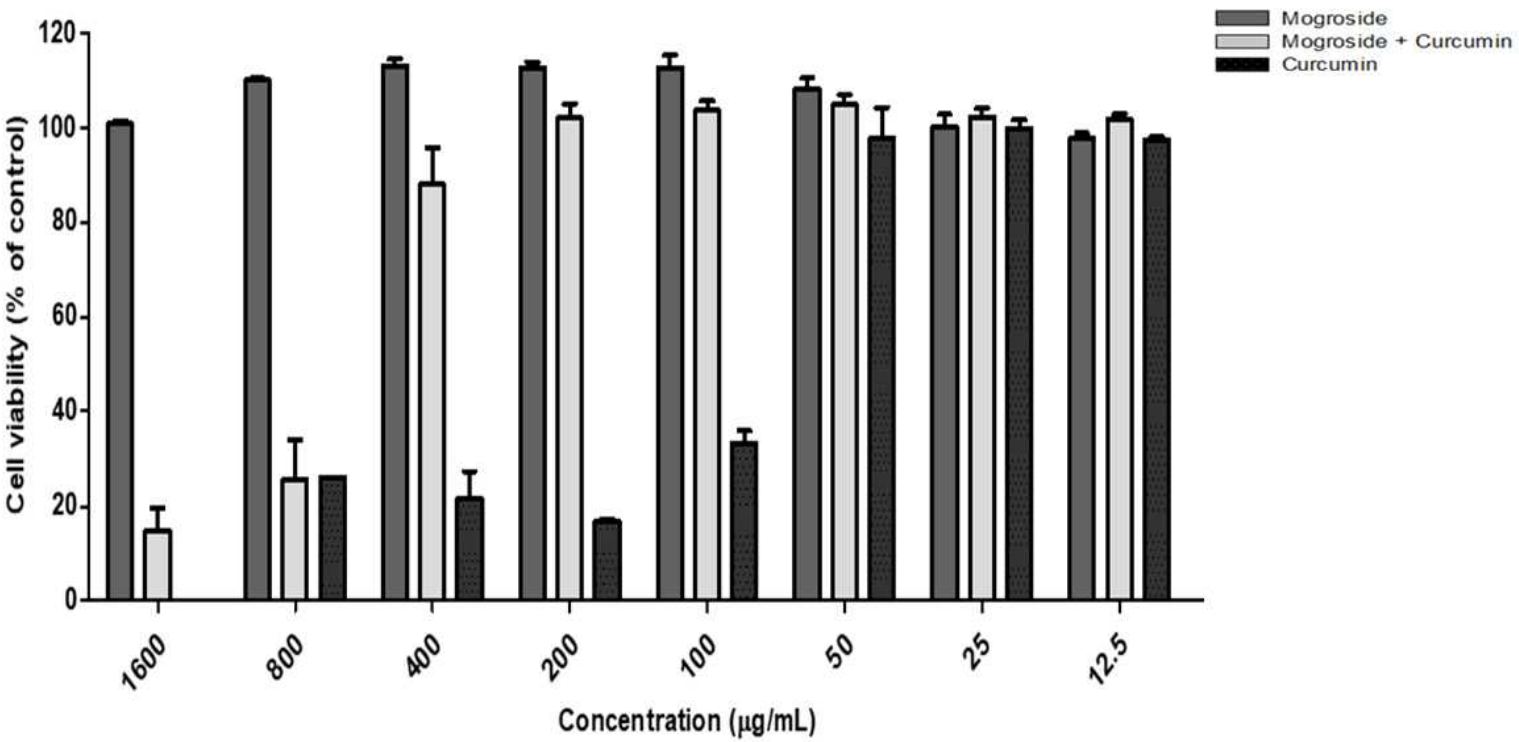


Fig. 16. Effect of mogroside V, curcumin, water soluble curcumin with mogroside V in cultured B16F10 cells. Data are expressed as percent change of the determination was made in triplicate.

7 Nitric oxide production inhibition of Mogroside V

Treatment of lipopolysaccharide (LPS) is known for stimulation of related factors such as NO and TNF-α by inflammatory reaction. Overexpressed NO or TNF-α have negative effects to human body causing systemic inflammation. However, proper amount of NO is considered as a key factor of congenital immunity. In this study, we conducted quantification analysis of NO production by treatment of curcumin, mogroside V and curcumin with mogroside V to LPS treatment group.

LPS treatment group significantly produced NO more than compared to untreated control group. We confirmed that indomethacine suppresed production of NO increased by LPS stimulation as a positive control. Mogroside V has effect for anti-inflammatory activity by reducing nitric oxide production. The effect of water soluble curcumin at different concentration are shown in (Fig. 17). The NO inhibitory activity was increased when concentration of water soluble was increased. At 200 μg/mL(approximately 17.86 μg/mL of curcumin), the NO inhibitory activity was similar as 12.5 μg/mL of curcumin. Although the effect is lower than when curcumin was dissolved in organic solvents, it also indicates that it is effective when curcumin is dissolved in water using mogroside V.

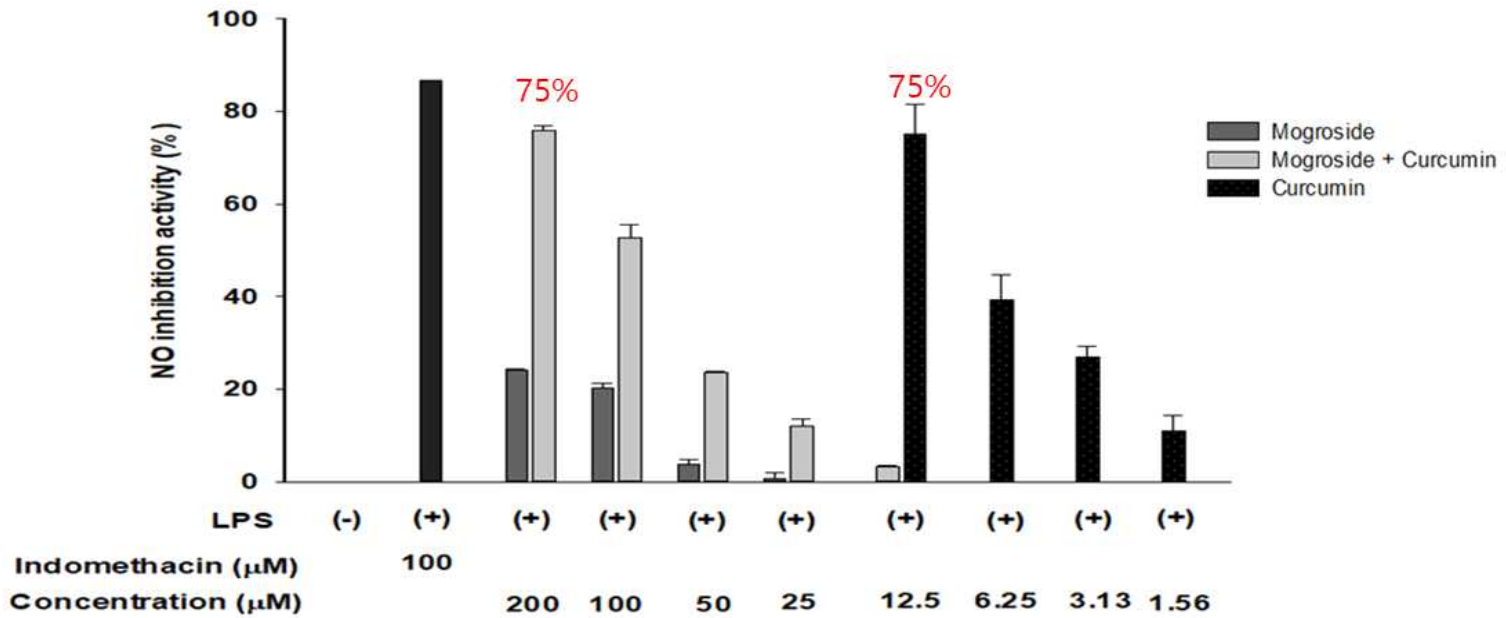


Fig. 17. Effect of mogroside V, curcumin, water soluble curcumin with mogroside V on the nitric oxide production by RAW267.4 cells. All data are presented as mean±standard deviation. Data are expressed as percent change of the nitric oxide production level relative to untreated control.

8 Effect of mogroside V on melanin content in B16F10 cells

To examine the melanin synthesis in the epidermal cell, curcumin, mogroside V and water soluble curcumin were treated on B16F10 melanoma cells. α-MSH stimulated the melanin synthesis. Experiments on the inhibition of melanin production were conducted in three ways. B16F10 cell treated with α-MSH shown increased melanin content extra-celluar 61% and intra-celluar 54% and celluar tyrosinase 235%. First, extra-celluar melanin content of cell treated with water soluble curcumin showed 65% inhibition activity at the 8.93 μg/mL melanin formation compared to control (treated with α-MSH). That's similar effect compare to normal curcumin 74% inhibition activity shown at the 10 μg/mL compared to control (Fig. 18).

Second, In intra-celluar melanin content the 4.46 μg/mL water soluble curcumin was higher inhibitory activity compared to 10 μg/mL of normal curcumin (Fig. 19). Intra-celluar and extra-celluar experiments confirmed that water soluble curcumin was effective in the melanin formation stage.

Based on the above experiment, melanin tyrosinase inhibition experiment was conducted, which greatly influences melanin formation. Normal curcumin and water soluble curcumin are shown similar celluar tyrosinase activity (Fig. 20). water soluble curcumin was showed 150%

inhibition activity at 8.93 μg/mL compare to control and normal curcumin was shown 168% inhibition activity at 10 μg/mL.

Through the above experiments, it was confirmed that water soluble curcumin is also effective in inhibiting melanin formation, such as curcumin dissolved in an existing organic solvent.

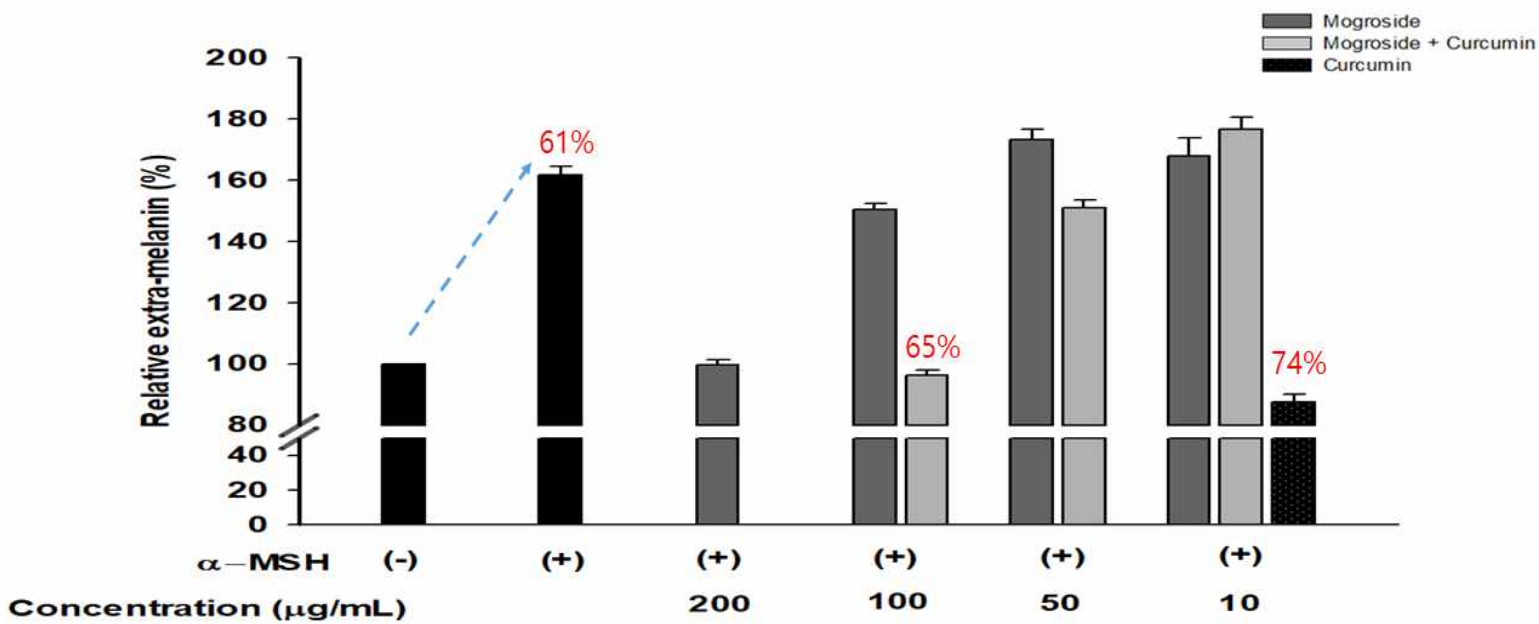


Fig. 18. Effect of mogroside V, curcumin, water soluble curcumin with mogroside V on extra-celluar melanin content in B16F10 cells. All data are presented as mean ± standard deviation.

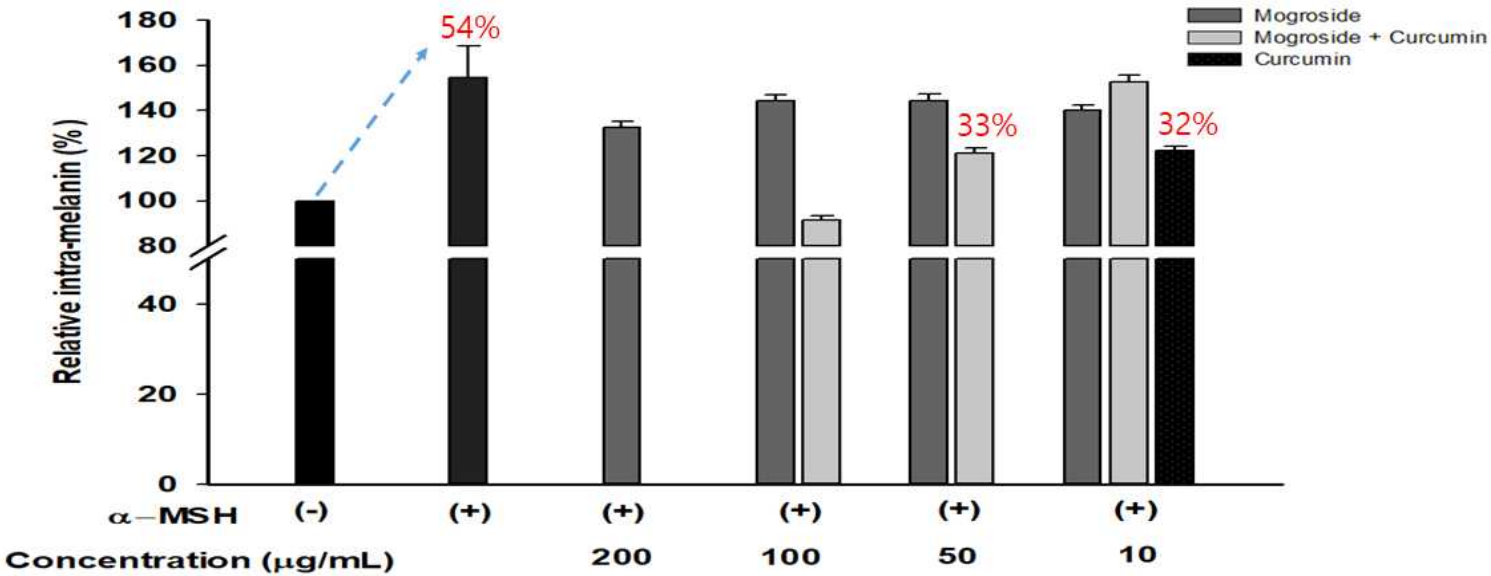


Fig. 19. Effect of mogroside V, curcumin, water soluble curcumin with mogroside V on intra-celluar melanin content in B16F10 cells. All data are presented as mean ± standard deviation.

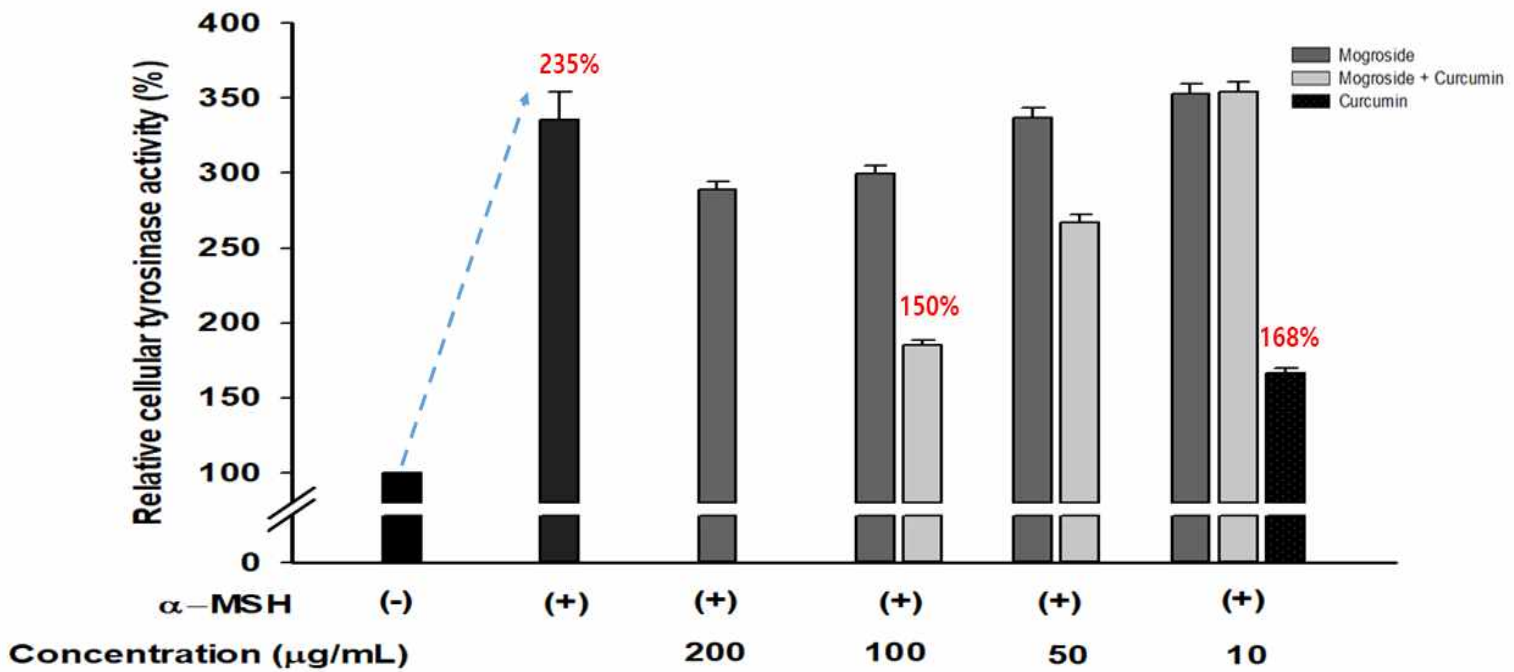


Fig. 20. Effect of mogroside V, curcumin, water soluble curcumin with mogroside V on celluar tyrosinase activity in B16F10 cells. All data are presented as mean ± standard deviation.

Conclusions

Mogroside V is the main component of the sweetener of luo han guo (*Siraitia grosvenorii*, Monk fruit). Its sweetness is approximately 300-times sweeter than sucrose, also has a low caloric value and low toxicity. But mogroside V is high price due to small amount of MV found in nature. To overcome this problem in this study we investigated efficient refining methods using HP20 and MPLC process.

Purified using HP-20, 55% purity of the monk fruit extract can be obtained and if extracts obtained through HP-20 are re-refined with MPLC, high purity mogroside V of 92% or more can be obtained.

In this study, I report mogroside V could increase solubility of the water insoluble compounds such as idebenone, bisdemetoxycurcumin, oleanolic acid, resveratrol, quercetin, taxol. especially in cucumin for the highest solubilized curcumin value was confirmed when 75 mg mogrosidse V was dissolved in 80% ethanol with curcumin 10 mg was the optimized conditions of solubilzed curcumin.

Antioxidant effect of curcumin in water sample is about 4 times lower than curcumin in ethanol. However, when curcumin, which was not soluble in water at all, when dissolved using mogroside V, the antioxidant effect was weak, but it was confirmed to exist. Probably the molecules of the mogroside V present in the area increased the viscosity therefore decreasing the mobility and also competing for the

interactions with molecules of curcumin, which reduced the interaction between curcumin and ferric iron(Fe^{3+}) molecules. Or mogroside V micelle formation can disturb curcumin's phenolic hydroxyl group interaction.

Water soluble curcumin has NO inhibition activity in RAW 264.7 cell 75% inhibitory activity showed at 17.86 μg/mL of water soluble curcumin, same 75% NO inhibitory activity showed at 12.5 μg/mL of curcumin. Although the effect is lower than when curcumin was dissolved in organic solvents, it also indicates that it is effective when curcumin is dissolved in water using mogroside V.

Extra-celluar melanin content of cell treated with water soluble curcumin showed 65% inhibition activity at the 8.93 μg/mL melanin formation compared to control (treated with α-MSH). That's similar effect compare to normal curcumin 74% inhibition activity shown at the 10 μg/mL compared to control. In intra-celluar melanin content 33% inhibition activity showed 4.46 μg/mL water soluble curcumin and it was higher inhibitory activity compared to 32% inhibition activity showed 10 μg/mL of normal curcumin. Intra-celluar and extra-celluar experiments confirmed that water soluble curcumin has inhibitory effect during melanin formation. Also melanin tyrosinase inhibition activity experiment was conducted, which greatly influences melanin formation.

Normal curcumin and water soluble curcumin are shown similar celluar tyrosinase activity. water soluble curcumin was showed 150%

inhibition activity at 8.93 μg/mL and normal curcumin was shown 168% inhibition activity at 10 μg/mL. It was confirmed that water soluble curcumin is also effective in tyrosinase inhibitory effect.

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Abstract in Korean

모그로사이드 V는 나한과로 알려진 luo han guo (Siraitia grosvenorii, monk fruit)의 주요 성분이다. 모그로사이드 V는 설탕 대체 감미 소재로써 설탕보다 300배 이상 단맛을 갖고 있고 이에 음식 소재로 사용된다. 그러나 모그로사이드 V는 가격이 비싸고 설탕대체 감미 소재 외 다른 특성의 연구가 부족한 상황이다. 이에 모그로사이드V의 특성 및 활용방법을 제시하고자 연구하였다. 모그로사이드V는 나한과에서 추출한뒤 HP20로 정제 할 경우 순도 55%이상의 모그로사이드V을 얻을수 있으며 이를 MPLC로 재정제 할 경우 순도 92%의 고순도 모그로사이드V을 추출 할수 있다. 기능성 연구 결과 이데베논(Idebenone), 레스베라트롤(Resveratrol), 올레아놀산(Oleanolic acid), 커큐민(Curcumin), 비스데메톡시커큐민(Bisdemethoxycurcumin), 타솔(Taxol), 쿼세틴(Quercetin) 의 수용성을 증가시켰으며 이는 천연 계면활성제로서의 모그로사이드 V 사용 가능성을 나타낸다. 수용성이 가장 증가한 커큐민을 이용해서 추가 기능성 연구를 하였다. 세포 독성과 항염증 작용은 쥐의 대식세포인 RAW 264.7 세포를 이용해 연구하였으며 모그로사이드 V을 이용해서 물에 녹인 커큐민은 17.86 μg/mL에서 75% 억제 능력을 보였다. 이는 12.5 μg/mL에서 75% 억제 능력을 보이는 기존의 유기용매에 녹인 커큐민에 비해 효과는 다소 낮아졌으나 물에 전혀 녹지 않던 커큐민을 모그로사이드V을 이용하여 녹였을 경우

항염 특성은 갖고 있는 것을 확인 하였다. 또한, 멜라닌 형성 억제 및 멜라닌 형성에 큰 영향을 미치는 Tyrosinase 억제 능력을 B16F10 세포를 이용해 연구하였으며 extra-celluar 에서는 물에 녹인 커큐민이 기존의 유기용매에 녹인 커큐민에 비해 비슷한 효능을 보였으며 intra-celluar 에서는 물에 녹인 커큐민이 기존의 커큐민보다 더 좋은 특성을 갖는 것을 확인하였다. Tyrosinase 억제 능력은 물에 녹인 커큐민과 유기용매에 녹인 커큐민의 특성이 비슷한 것으로 확인되었다.