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Inhibition of Adenovirus 36 Replication and Lipid Accumulation by *Distylium racemosum*

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Abstract

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Obesity is a worldwide disease and one of the major risk factors. Virus among many factors can lead to obesity. Adenovirus 36 (Ad-36) is the only adipogenic virus linked with human obesity. Nevertheless, there is no drug to treat both Ad-36 infection and obesity associated with virus. For the precedent study on anti-oxidant and anti-cholesterol test, *Distylium racemosum*, *Quercus salicina Blume* and *Raphiolepis indica var* were selected. In this study, physiological effects such as the anti-oxidant,

anti-cholesterol and anti-lipid effects of three extracts were analyzed, compared and established the inhibition of Ad-36 replication. *D. racemosum*, *Q. salicina Blume* and *R. indica var* all had high anti-oxidant effects no less than 80% and they showed abilities which were similar to quercetin of the positive control group. Total phenolic contents were confirmed in the three medical plants and associated with 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity results. Total phenolic contents of *D. racemosum*, *Q. salicina Blume* and *R. indica var* were 134.53 mg GAE/g extract, 85.20 mg GAE/g extract and 65.20 mg GAE/g extract, respectively. Particularly, *D. racemosum* (50 ㎍/ml) inhibited lipid accumulation on 3T3-L1 preadipocyte. *D. racemosum* inhibited adipocyte differentiation through suppression of regulator peroxisome proliferator-activated receptor-ɣ (PPAR ɣ) genes and adipocyte-specific genes such as adipocyte protein 2 (aP2). *D. racemosum* inhibited replication of Ad-36 at 50 ㎍/ml of concentration. Therefore, the extract of *D. racemosum* could be a candidate for development of anti-Ad-36 and anti-obesity drugs.

Key words : *D. racemosum,* Ad-36, anti-lipid, anti-oxidant, anti-cholesterol

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Ⅰ. Introduction

Obesity is defined as having an abnormal or excessive amount of body fat accumulation and is one of the major risk factors for chronic diseases including diabetes, cardiovascular disease and cancer (WHO, 2014). At least 2.8 million of the world popularity are dying due to obesity and it has reached epidemic proportions worldwide (WHO, 2014). Therefore, obesity is the most serious public health problem declared from the World Health Organization (WHO) (Baek *et al*., 2014).

Obesity is occurred by many causes. Recent studies have been made actively about correlation between virus infection and obesity. Viruses such as canine distemper virus, Rous-associated virus 7, Borna virus and SMAM-1 cause obesity in animals, and nearly every known Adenovirus 36 (Ad-36) is associated with obesity in humans (Vangipuram *et al*., 2004). Adenoviruses are contained of 50 different types and causes acute upper respiratory infections. Adenovirus 36 was first isolated from the slurry of a six year old girl affected by diabetes and enteritis in 1978 (Dhurandhar, 2001). Eleven percent of non-obese people of American adults, 30% of obese people of American adults, and 30% of obese children of South Korea were reported that they were infected with Ad-36 (Atkinson, 2011). Fat cells infected with Ad-36 have been known for the differentiation to more fat cells and more lipid accumulation in cells. When E4orf1 gene of Ad-36 was inserted into preadipocyte, adipogenic transcription factors were stimulated (Suplicy *et al*., 2009). Group infected with Ad-36 has shown significantly low serum cholesterol and triglyceride levels compared with the control group (Pasarica *et al*., 2012).

Obesity is generated by adipocyte differentiation and fat accumulation. Adipocyte differentiation is controlled by specific gene expression (Song *et al.*, 2013). Major specific genes associated with fat accumulation are peroxisome proliferator-activated receptor-ɣ (PPARɣ) and CCAAT/enhancer binding protein-α (C/EBPα). Especially, adipogenesis are mainly controlled by the PPARɣ, belonged to nuclear receptor superfamily (Zhang *et al.,* 2012: García-Alonso *et al.*, 2013). PPARɣ induces the expression fatty acid binding protein such as adipocyte protein 2 (aP2) as well as many other genes (Park *et al.*, 2012).

According to the research on other causes of obesity, fatty tissue accumulated on obesity is the result of oxidative stress and reactive oxygen species (ROS) which are causes of chronic illness (Lamichhane *et al.*, 2014). ROS such as superoxide anion ($\cdot O_2$), hydroxylradical (·OH) and hydrogen peroxide (H_2O_2) are produced by normal metabolic processes or exogenous factors, and they can easily store lipid peroxides (Nandita *et al.,* 2004). Antioxidant enzymes such as super-oxide dismutase (SOD), catalase(CAT), glutathione peroxidase, and small molecules substances such as glutathione are prepared to defend the excessive ROS in the body (Bayati *et al.*, 2011). However, the excessive ROS and low antioxidant defenses were leading to structural and functional changes in the molecular (Kumarappan *et al.*, 2012). Antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propylgallate (PG) showed powerful free radical scavenging effects, but appear side effects such as liver damage and carcinogenicity (Chipiti *et al.*, 2013). Recently, studies on development of antioxidants using natural materials have been increased. Natural antioxidants have many useful effects like anti-viral, anti-mutagenic, anti-inflammatory, anti-cancer and hepatoprotective effects (Ali *et al.*, 2013).

Jeju Island of the Korea has a valuable ecosystem, and is known for the variety and richness of plants and over 7800 species of them have been classified to date (Moon *et al.*, 2010). Therefore, natural extracts from Jeju Island were tested by anti-cholesterol and anti-oxidant screening test. Three natural extracts among them were selected as cholesterol inhibitors and anti-oxidants. The extracts are extracted from the leaves of *Distylium racemosum, Quercus salicina Blume* and *Raphiolepis indica var* which are ones of oriental medical plants grown wildly in Jeju. *D. racemosum* is known for anti-oxidant effects (Ko *et al.*, 2011). *Q. salicina Blume* is known for anti-inflammatory, anti-edemic, diuretic and litholytic activities (Song *et al.*, 2013). *R. indica var* has been a decorative form of art. However, scientific data on biological and physiological effects of those are insufficient.

This study investigated and compared anti-oxidant and anti-cholesterol effects of extracts from *D. racemosum, Q. salicina Blume* and *R. indica var.* Quercetin was used as single component and positive control. Quercetin is well known as scavenge superoxide radicals and protect from lipid peroxidation (Raju *et al.*, 2000). The effect of extracts on the adipocyte differentiation of 3T3-L1 cells were investigated by measuring lipid accumulation and the expression major adipogenic transcriptional factor such as PPARγ and adipocyte-specific genes such as aP2. Moreover, Ad-36 replication in A549 cell line treated with the extracts was examined by comparing Ad-36 hexon DNA expression. Inhibition of Ad-36 by medicinal herbs having anti-oxidant, anti-cholesterol and anti-lipid effects, might be used for drug development to prevent obesity and to treat viruses.

Ⅱ. Materials and Methods

1. Extraction of medicinal plants

Ethanol extraction approach is employed to extract medical plant materials which are five hundred forty eight species of medical plants from Jeju, Korea. The fresh leaves of them were collected, washed with tap water, air dried, homogenized to a fine powder and stored in air-tight containers. The air dried powder (100g) was extracted with ethanol (40-60℃) in a extractor for 18-20 hours and solution was evaporated to dryness under reduced pressure and controlled temperature by using roto evaporator. The extract was stored in a refrigerator at 4℃ in air-tight bottles until further use. The concentration of extracts was prepared to be 10mg/ml in ethanol and used. Quercetin was used as positive control. The extracts were tested by anti-cholesterol and anti-oxidant screening test.

2. Cholesterol adsorptivity

Cholesterol adsorptivity of them was measured by total cholesterol kit (Asan-Pharm, Seoul) designed by enzyme reaction. Briefly, 100 μl of different concentration of nature extracts ranging from 0.1 to 10 mg/ml were mixed with 1.8 mg/ml cholesterol (Sigma-aldrich, USA) in EtOH, and reacted at 20℃ for 20 minutes. 50 μl of 0.1 M hexadecyl-trimethylammonium bromide (Sigma-aldrich, USA) was added and centrifuged at 4℃ for 10 minutes at 15,000 rpm. After supernatant was collected, it was reacted with enzyme solution at 37℃ for 5 minutes. Observance density (OD) was measured at 500 nm using spectrophotometer.

3. DPPH radical scavenging activity

The extracts were diluted to 0.1, 0.5, 1, 5 mg/ml concentrations in each 70% EtOH. 10 μl of samples was added to 190 μl of 200 μM DPPH (Sigma-aldrich, USA) dissolved in ethanol. After reacted at 37℃ for 30 minutes, observance density (OD) was measured at 550 nm using ELISA reader. The antioxidant activity of the extracts was compared to that of quercetin as an antioxidant standard.

4. Total phenolic contents

Total phenolic contents were determined using the Folin-Denis method. The extract solution or gallic acid (Sigma-aldrich, USA) standard (50 μL) was mixed with 1.65 ml of D.W. and 100 μL of Folin-Denis reagent (Sigma-aldrich, USA). After incubation for 5 minutes, 200 μL of 1 N Na_2CO_3 was added and the solution was allowed to stand for 2 hours at room temperature. Absorbance of the resulting blue complex was measured at 750 nm using spectrophotometer. Total phenolic content was reported as mg of gallic acid equivalents per gram extracts (mg GAE/g extracts).

5. Cell viability assays

3T3-L1 preadipocyte cells (Korean Cell Line Bank) were cultured in Dulbecco's modified Eagle's medium (DMEM) high glucose containing a 10% bovine calf serum (BCS), 1% penicillin and streptomycin for proliferation at 37℃ and 5% CO_2. Human epithelial (A549) cells (Korean Cell Line Bank) were cultured in Dulbecco's modified Eagle's medium (DMEM) high glucose containing a 10% fetal bovine serum (FBS), 1% nonessential

amino acids, 1% penicillin and streptomycin for proliferation at 37℃ and 5% CO_2. After cell was counted, it was diluted to 1×10^5 cell/ml. Then, it was pipetted to 96 well plate each 100 ㎕. It was incubated at 37℃ and 5% CO_2 for one night. The extracts from nature extracts were diluted 2, 10, 50, 100, 200 ㎍/ml concentrations in each Ethanol, it was pipetted each 100 ㎕. It was shaked at 150 rpm for 5 minutes and incubated at 37℃ and 5% CO_2 for 3 day. 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) solution (Amresco, Korea) concentrated on 5 mg/ml in PBS was pipetted each 20 ㎕. After shaked at 150 rpm for 5minutes, it was incubated at 37℃ and 5% CO_2 for 2 hours. Medium were deleted completely. DMSO were pipetted each 200 ㎕ and it was shaked at 150 rpm for 5 minutes. Observance density (OD) was measured at 560 nm using ELISA reader.

6. Differentiation of 3T3-L1 preadipocytes

3T3-L1 preadipocytes were grown in Dulbecco's modified eagle medium (DMEM) high glucose with 10% bovine calf serum (BCS) and 1% penicillin-streptomycin (PS). At 2 day post confluence, cell differentiation was induced with 10% fetal bovine serum (FBS), 10 ㎍/ml insulin (INS), 1 ㎛M dexamethasone (DEX), 0.5 ㎛M isobutylmethylxanthine (IBMX) and 1% PS. After 3 days, cells were then maintained in DMEM high glucose with 10% FBS, 10 ㎍/ml INS and 1% PS for 4 additional days. This medium was changed every 2 days. The adipocytes were treated into the culture medium containing the extracts at 2 day. Cells were treated with 0, 2, 10 and 50 ㎍/ml of extracts. After treatment with extracts for 7 days, the 3T3-L1 adipocytes were staining and RT-PCR.

7. Oil Red O staining of 3T3-L1 adipocytes

3T3-L1 cells were incubated in cell culture media containing with various concentration of the extracts, washed twice with phosphate-buffered saline (PBS), and then fixed in 10% formaldehyde prepared for 1 hour at room temperature. The cells were washed with distilled water (DW) three times, and were stained with the oil red O (Sigma-aldrich, USA) solution for 2 hours at room temperature. The cells were then washed in DW three times for 1 hour each time. All photographs were taken using a microscope.

8. Reverse transcription-PCR (RT-PCR)

RNA was isolated by accuzol ^{TM} RNA extraction solution (Bioneer,Korea). Isolated RNA was reverse transcribed to cDNA for 60 minutes at 42°C and then 10 minutes at 72°C by RT-PCR (Bioneer,Korea). The primers were designed following as: GAPDH forward - GCT AGG ACT GGA TAA GCA GGG, GAPDH reverse - GAT GGG CTT CCC GTT GAT GA, PPARɣ forward - GTG AGA CCA ACA GCC TGA CG, PPARɣ reverse - ACA GAC TCG GCA CTC AAT GG, aP2 forward - GAA ATC ACC GCA GAC GAC AG, aP2 reverse - AAC TCT TGT GGA AGT CAC GCC. The amplification cycles were 94°C for 1 minutes, 56°C for 1 minutes and 72°C for 1 minutes. After 35 cycles, PCR products were separated by electrophoresis on 1.5% agarose gel for 25 minutes at 100 V. Gels were stained with ethidium bromide visualized by Alpha Innotech imaging system.

9. Inhibition of Adenovirus 36 replication

Human epithelial (A549) cells were infected with 1 MOI of adenovirus 36 for 2 hours. After infection, the cells were cultured in complete DMEM containing 10% fetal bovine serum and 1% penicillin-streptomycin in an incubator with the condition of 37°C and 5% CO_2. After 48 hours, the infected cells were collected for further analyses. Genomic DNA was extracted using an *AccuPrep* Genomic DNA extraction Kit (Bioneer, Korea). The primers were designed following as: GAPDH forward primer - CCC ACC ACA CTG AAT CTC CC, GAPDH reverse primer - CTC ACC TTG ACA CAA GCC CA , Ad-36 hexon forward primer - ATG GCC AGC TAC TTT GA, Ad-36 hexon reverse primer - TGA GGT TCT GGC TGG AAA GT. The amplification cycles were 94°C for 1 minutes, 56°C for 1 minutes and 72°C for 1 minutes. After 35 cycles, PCR products were separated by electrophoresis on 1.5% agarose gel for 25 minutes at 100 V. Gels were stained with ethidium bromide visualized by Alpha Innotech imaging system.

10. Statistical analysis

Statistical analysis of data was performed with ANOVA followed by *P*-values less than 0.05 were considered significant.

Ⅲ. Results

1. Cholesterol reduction effects of the natural extracts

Three extracts among them were selected because of high biochemical activity. The medical plants are *Distylium racemosum, Quercus salicina Blume* and *Raphiolepis indica var* which are ones of oriental medical plants grown wildly in Jeju.

Cholesterol adsoptivity of the extracts (10 mg/ml) from *D. racemosum*, *Q. salicina Blume, R. indica* and Quercetin were 69%, 39%, 32% and 84%, respectively. Cholesterol adsorptivity of the extracts from *D. racemosum*, *Q. salicina Blume* and *R. indica va* were not very high compared to that of quercetin, the single component. Cholesterol adsoptivities by the all extracts were gradually increased depending on concentration. Cholesterol adsorptivity of the extracts from *D. racemosum* and Quercetin was dramatically increased compared to those of *Q. salicina Blume* and *R. indica var* through dose-dependent increase. Cholesterol adsoptivity of the Extract from *D. racemosum* was highest among the nature extracts (Fig. 1).

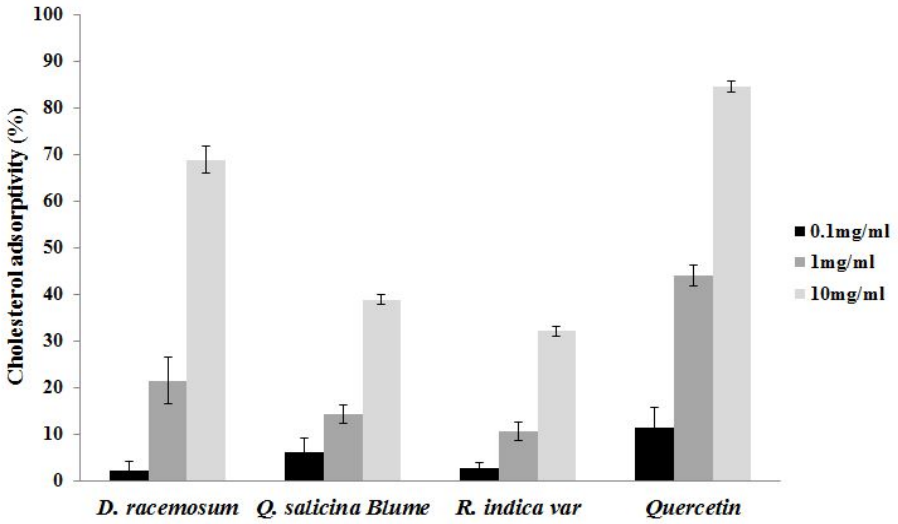


Figure 1. Cholesterol reduction effects of natural extracts from *D. racemosum, Q. salicina Blume, R. indica var* and Quercetin. Cholesterol adsoptivity of *D. racemosum*, *Q. salicina Blume, R. indica var* and Quercetin were 69%, 39%, 32% and 84%, respectively, at 10 mg/ml of concentration. Quercetin as material and positive control material was physiologically active. *D. racemosum* among the natural extracts showed the highest cholesterol reduction effects. ($p<0.05$)

2. Anti-oxidant effects of the natural extracts

Anti-oxidant effects of the extracts were tested by DPPH radical scavenging test. Anti-oxidant effects of the extracts 5 mg/ml from *D. racemosum*, *Q salicina Blume, R. indica var* and Quercetin were 95%, 92%, 90% and 86%, respectively. Anti-oxidant effects by the all extracts were gradually increased depending on concentration. Anti-oxidant effects of the extracts were increased that of Quercetin which is one of representative anti-oxidant materials (Fig. 2). However, DPPH radical scavenging activities of 0.5 mg/ml *D. racemosum* and that of 0.1 mg/ml Quercetin were over 30%. Anti-oxidant effect of *D. racemosum* was highest among them.

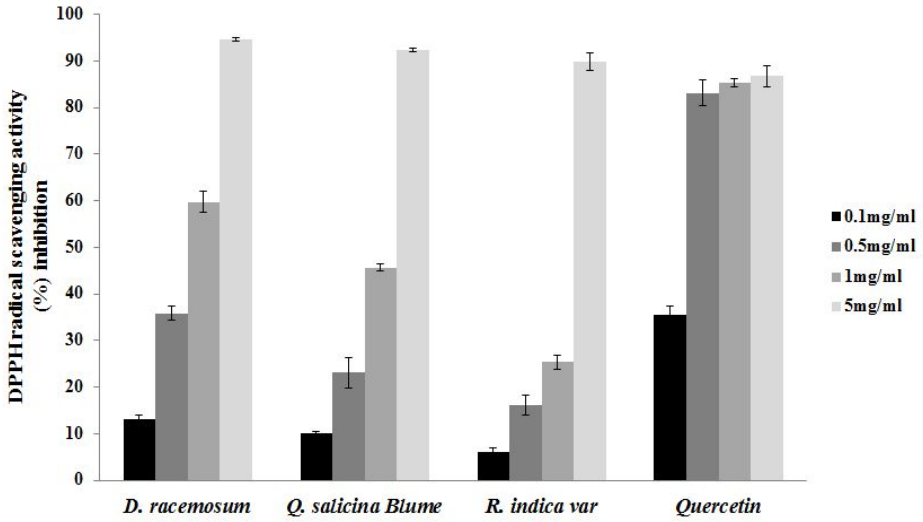


Figure 2. DPPH radical scavenging activities of natural extracts from *D. racemosum, Q. salicina Blume, R. indica var* and Quercetin. DPPH radical scavenging activities of the extracts 5 mg/ml from *D. racemosum*, *Q. salicina Blume, R. indica var* and Quercetin were 95%, 92%, 90% and 86% respectively. Quercetin is one of representative anti-oxidant materials and used as positive control. *D. racemosum* among the natural extracts showed the highest DPPH radical scavenging activity. ($p<0.05$)

3. Total phenolic contents of the natural extracts

Total phenolic contents of the extracts were measured by the Folin-Denis method and associated with DPPH radical scavenging activity results. Total phenolic contents of from *D. racemosum*, *Q salicina Blume* and *R. indica var* were 134.53 mg GAE/g extract, 85.20 mg GAE/g extract and 65.20 mg GAE/g extract, respectively (Table 1). Total phenolic content of *D. racemosum* was most among them and showed the strongest DPPH radical scavenging activity.

Table 1. Total phenolic contents of natural extracts from *D. racemosum, Q. salicina Blume* and *R. indica var*

Extracts	Total phenolic contents (mg GAE/g extract)
D. racemosum	134.5 3± 3.11
Q. salicina Blume	85.20 ± 2.00
R. indica var	65.20 ± 1.74

D. racemosum among the natural extracts contained the most total phenolic content. ($p<0.05$)

4. Cell viability of the natural extracts on 3T3-L1 and A549 cells

Cell viability of the extracts was determined by MTT assay on 3T3-L1 cells and A549 cells. The cells were treated with various concentrations (0 ~ 200 μg/ml) of quercetin, *D. racemosum, Q. salicina Blume and R. indica var.* The extracts of *D. racemosum* and *Q. salicina Blume* showed cell toxicity over 100 μg/ml concentration on 3T3-L1 cells. The extracts of *R. indica var.* showed cell toxicity over 200 μg/ml concentration on 3T3-L1 cells. The concentration of Quercetin to not affect cell viability is under 10 μg/ml concentration on 3T3-L1 cells. Extracts of *D. racemosum* and Quercetin showed cell toxicity over 100 μg/ml concentration on A549 cells. Extracts of *Q. salicina Blume* and *R. indica var* showed cell toxicity over 200 μg/ml concentration on A549 cells (Fig. 3, 4).

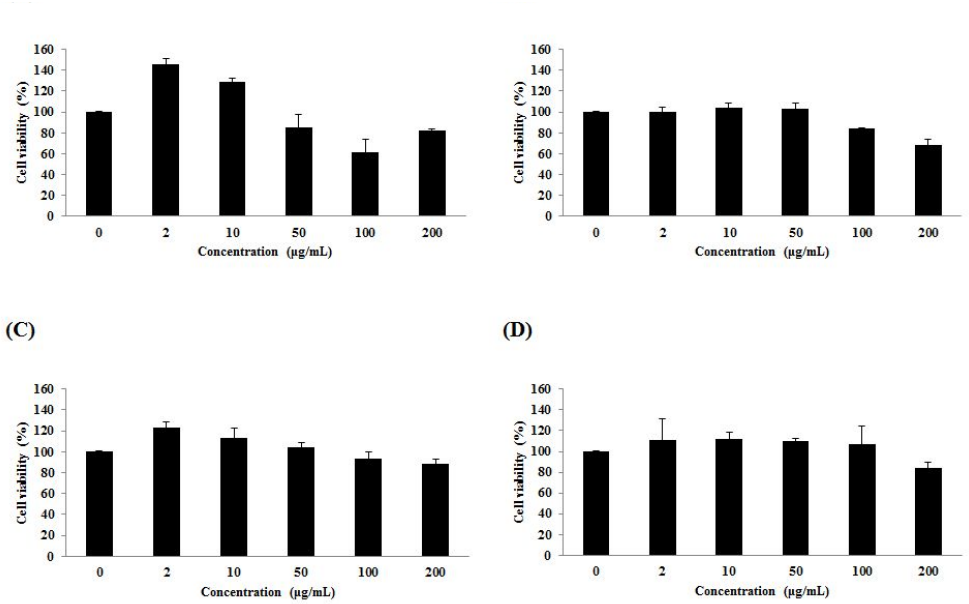


Figure 3. Cell viability effects of natural extracts from *D. racemosum, Q. salicina Blume, R. indica var* and Quercetin in 3T3-L1 cells. 3T3-L1 cells were treated with various concentrations (0 ~ 200 μg/ml) of quercetin, *D. racemosum, Q. salicina Blume* and *R. indica var.* Cell viability was measured by the MTT assay after 72 hours. The concentration of the three natural extracts and Quercetin to not affect cell viability is 50 μg/ml and 10 μg/ml, respectively. Therefore, the concentration is determined as maximum concentration in every experiment. (A) Quercetin, (B) *D. racemosum*, (C) *Q. salicina Blume*, (D) *R. indica var.* ($p<0.05$)

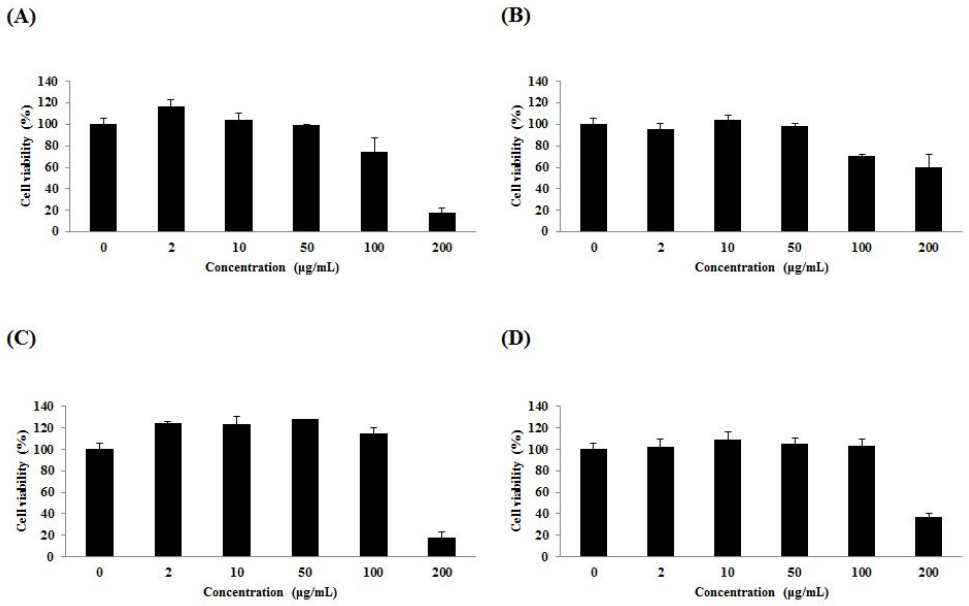
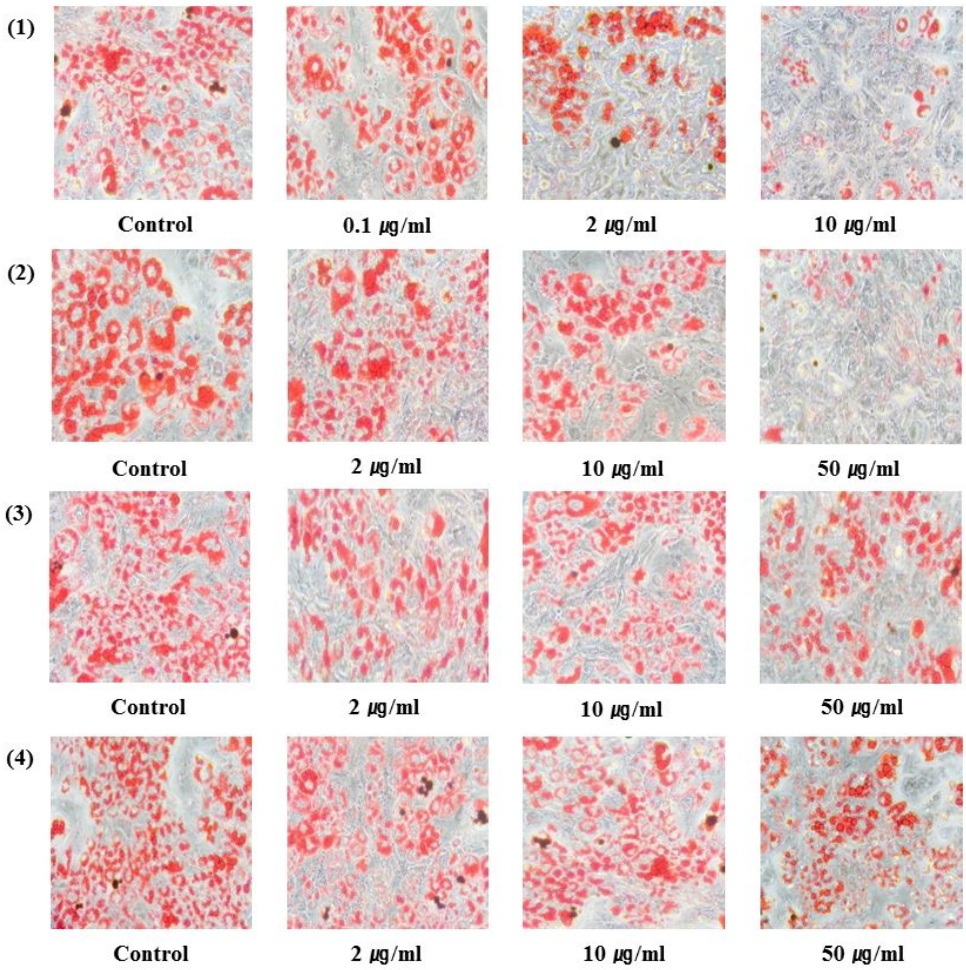


Figure 4. Cell viability effects of natural extracts from *D. racemosum, Q. salicina Blume, R. indica var* and Quercetin in A549 cells. A549 cells were treated with various concentrations (0-200㎍/ml) of quercetin, *D. racemosum, Q. salicina Blume* and *R. indica var.* Cell viability was measured by MTT assay after 72 hours. The concentration of the three natural extracts and Quercetin to not affect cell viability is 50 μg/ml. Therefore, the concentration is determined as maximum concentration in every experiment. (A) Quercetin, (B) *D. racemosum*, (C) *Q. salicina Blume*, (D) *R. indica var.* ($p<0.05$)

5. Inhibitory effect on adipogenesis and lipid accumulation by the natural extracts

To examine the anti-lipid effects of *D. racemosum*, *Q salicina Blume, R. indica var* and Quercetin, 3T3-L1 preadipocytes were treated with the extracts for 7 days, and stained with Oil red O. Adipogenesis and lipid accumulation in the cells was inhibited over 50% at 10 μg/ml Quercetin and 50 μg/ml *D. racemosum.* Adipogenesis in the cells were not inhibited by the extracts of both *Q. salicina Blume* and *R. indica var.* These results indicate that the extract from *D. racemosum* might be used effectively for development of anti-lipid drugs. The extracts from *Q. salicina Blume* and *R. indica var* showed no anti-lipid effects (Fig. 5).

A.



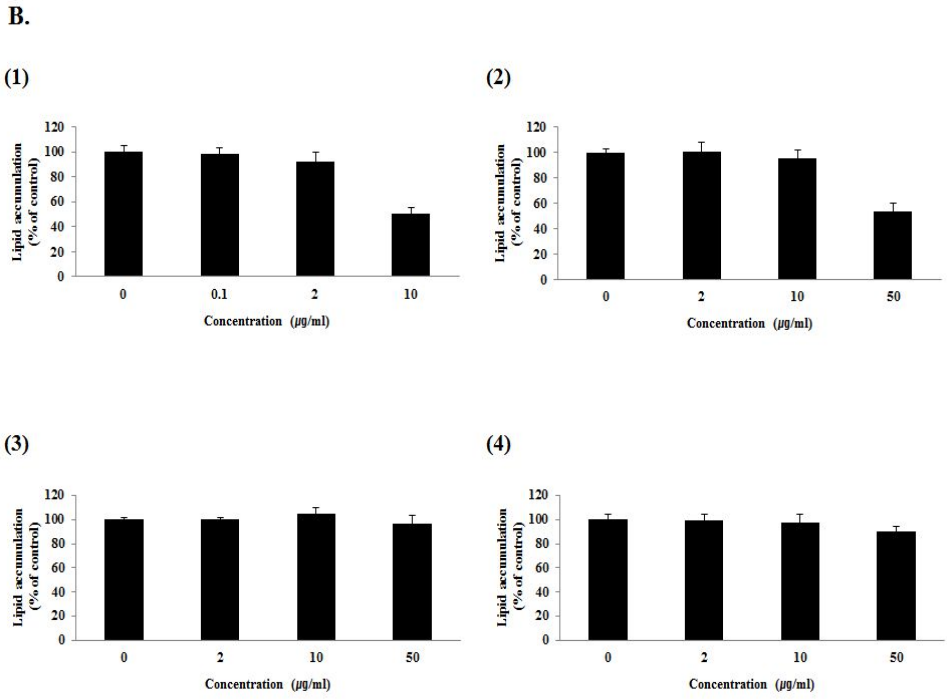


Figure 5. Inhibitory effects of lipid accumulation by the natural extracts in 3T3-L1 cells. (A) Photomicrographs of 3T3-L1 cells. Confluent 3T3-L1 preadipocytes were differentiated into adipocytes in the medium containing various concentrations of Quercetin, *D. racemosum, Q. salicina Blume* and *R. indica var* for 7days. The cells were stained with Oil-red O to measure lipid accumulation on day 7. (B) Isopropyl alcohol was used to elute the Oil-red O. Lipid accumulation was decreased in the cell treated with Quercetin and *D. racemosum,* significantly. ($p<0.05$) (1) Quercetin, (2) *D. racemosum*, (3) *Q. salicina Blume*, (4) *R. indica var.*

6. Inhibition of adipogenic specific genes expression by the natural extracts

To examine whether the expression of adipogenic transcription factors is inhibited by the extracts from *D. racemosum*, *Q salicina Blume, R. indica var* and Quercetin, 3T3-L1 preadipocytes were treated with various concentration of the extracts and incubated for 7 days. The expression of PPAR-γ gene was inhibited from 10 μg/ml Quercetin and 50 μg/ml *D. racemosum*. The expression of aP2 gene was inhibited from 2 μg/ml quercetin and 50 μg/ml *D. racemosum*. PPARγ and aP2 genes were inhibited by the extract of *D. racemosum* and Quercetin in dose-dependent manner. However, of PPARγ and aP2 gene were not inhibited by the extracts of both *Q. salicina Blume* and *R. indica var. D. racemosum* inhibited adipocyte differentiation through suppression of regulator peroxisome proliferator-activated receptor-γ (PPARγ) genes and adipocyte-specific genes such as adipocyte protein 2 (aP2) (Fig. 6).

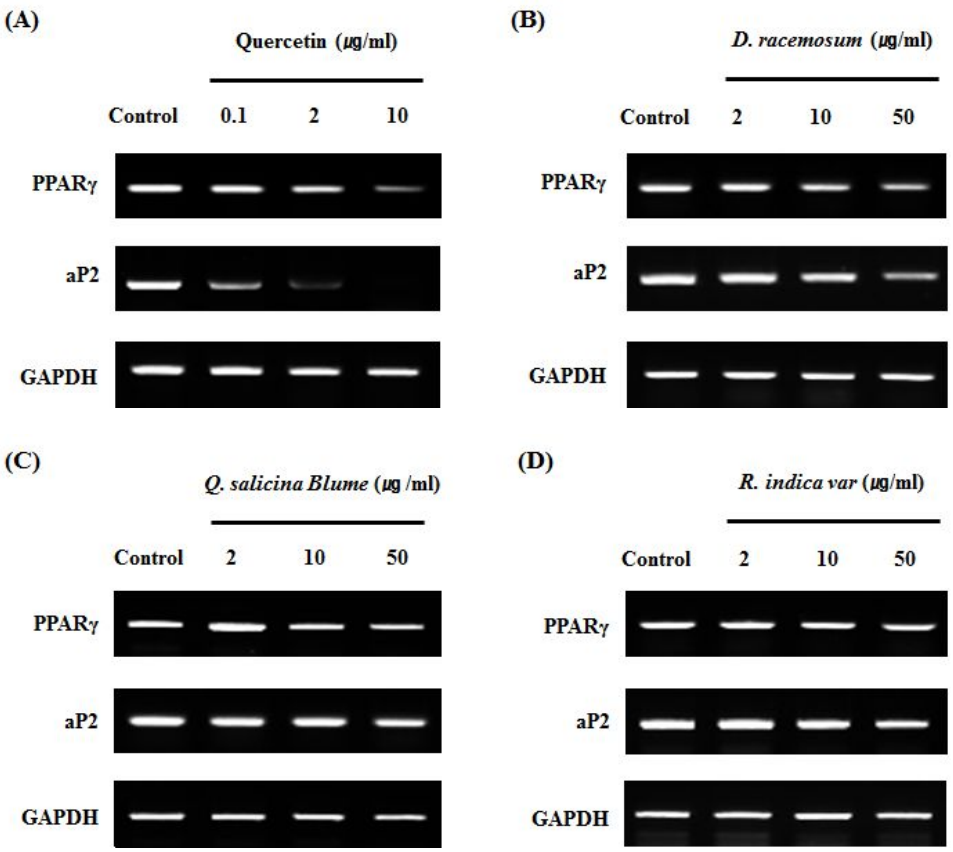


Figure 6. Inhibitory effects of transcriptional factors and adipocyte-specific genes by the natural extracts. Confluent 3T3-L1 preadipocytes were differentiated into adipocytes in medium containing various concentrations of Quercetin, *D. racemosum, Q. salicina Blume* and *R. indica var* for 7days. On day 7, PPARγ and aP2 genes which are transcriptional factors and adipocyte-specific genes, and GAPDH as cell control gene was analyzed by RT-PCR. PPARγ and aP2 gene transcriptions were inhibited in the cell treated with Quercetin and *D. racemosum,* significantly. ($p<0.05$) (A) Quercetin, (B) *D. racemosum*, (C) *Q. salicina Blume*, (D) *R. indica var.*

7. Inhibitory effects of Ad-36 replication by the natural extracts

To examine whether Ad-36 replication is inhibited by the natural extracts, A549 cells were infected with Adenovirus 36 for 2 hours and were cultured for 48 hours in the cell culture media containing the natural extracts. After 48 hr, hexon gene of Adenovirus 36 and GAPDH as cell control gene was analyzed by RT-PCR. Hexon gene of Adenovirus 36 was inhibited in the cell treated with 10 μg/ml Quercetin and 50 μg/ml *D. racemosum,* significantly. Hexon gene of Adenovirus 36 was inhibited by the extract of *D. racemosum* and Quercetin in dose-dependent manner. However, Hexon gene of Adenovirus 36 was not inhibited by the extracts of both *Q. salicina Blume* and *R. indica var. D. racemosum* inhibited the replication of Adenovirus 36 in the cells (Fig. 7).

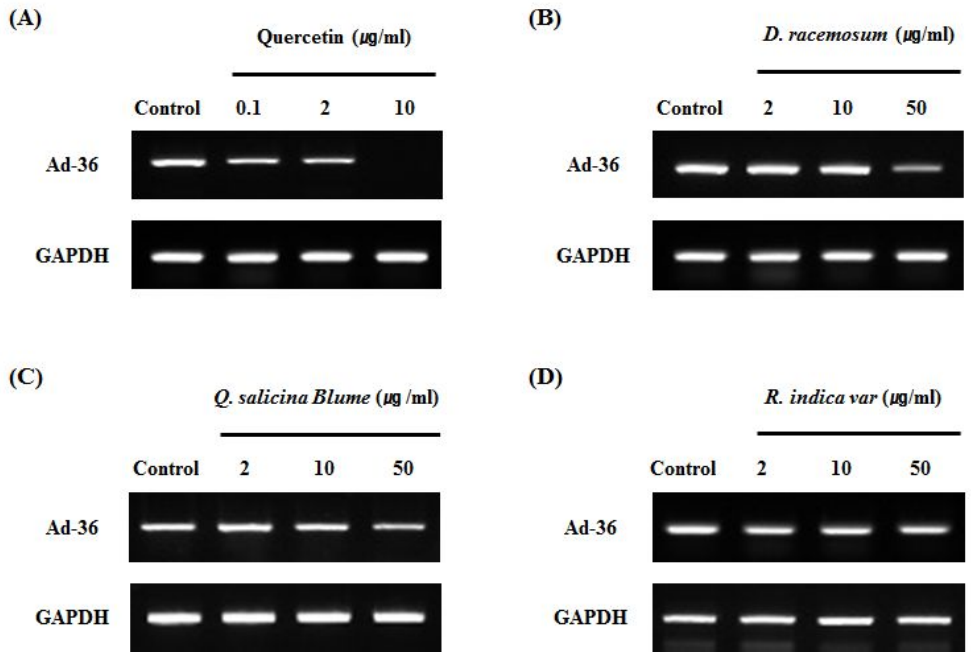


Figure 7. Inhibitory effects of Adenovirus 36 replication by the natural extracts. A549 cells were infected with adenovirus 36 for 2 hours. After infection, the cells were cultured for 48 hours in the cell culture media containing the natural extracts. After 48 hours, hexon gene of Adenovirus 36 and GAPDH as cell control gene was analyzed by PCR. Hexon gene of Adenovirus 36 was inhibited in the cell treated with Quercetin and *D. racemosum,* significantly. ($p<0.05$) (A) Quercetin, (B) *D. racemosum*, (C) *Q. salicina Blume*, (D) *R. indica var.*

Ⅳ. Discussion

Obesity and virus infection are one of serious problems in public health of human and animal in the world. Many studies have been performed using natural sources to overcome the obesity and the disease caused by virus. Particularly, human adenovirus 36 (Ad-36) is associated with obesity in human. In order to develop the anti-cholesterol, anti-lipid and anti-viral drugs, obesity cell model using human adenovirus 36 (Ad-36) has been valuable. Recently, development of new drug using natural extracts has been shedding new light. There are over 7800 species of plants in Jeju island of the Korea where is surrounded with special environment. This study selected natural extracts which have effect to anti-oxidant and anti-cholesterol in 548 species of medical plants from Jeju island. The 3 plants showing anti-oxidant and anti-cholesterol effects were *D. racemosum*, *Q. salicina Blume* and *R. indica var.*

Particularly, cholesterol adsoptivity, Anti-oxidant effect by DPPH radical scavenging test of the extract from *D. racemosum* were highest among the nature extracts. The effects of the extract from *D. racemosum* were identical to those of quercetin. These effects were resulted from total phenolic contents, which were associated with anti-oxidant effect. Total phenolic contents of from *D. racemosum*, *Q salicina Blume* and *R. indica var* were 134.53 mg GAE/g extract, 85.20 mg GAE/g extract and 65.20 mg GAE/g extract, respectively. Total phenolic content of *D. racemosum* was most among them and showed the strongest DPPH radical scavenging activity.

Recently, studies have been performed actively for anti-obesity effects using 3T3-L1 preadipocyte that is used as a model for the adipocyte

differentiation which is made more complicated by the adipogenic transcription factors (Park *et al*., 2011: Yang *et al*., 2014). Accumulated fat were significantly inhibited in 3T3-L1 preadipocytes treated with 50 μg/ml *D. racemosum* compared to that in the cells treated only with differentiation inducers. In addition, The expression of PPARγ, major transcription factors, and aP2, adipocyte-specific genes, were suppressed in the cells treated with 50 μg/ml *D. racemosum*. PPARγ was constitutes a expression of adipocyte-specific genes through a master regulator of adipocyte differentiation such as aP2 (Park *et al*., 2014). Kim *et al*. (2012) showed that *Citrus aurantium* flavonoid suppressed adipogenesis by down regulation of PPARγ and C/EBPα that are relevant to lipid accumulation and lipid metabolism. Song *et al*. (2013) showed that the anti-obesity effects of the extracts of blueberry peel on 3T3-L1.

Many factors and contributions lead to obesity, but researchers have been pondering the infection obesity for years. Human Ad-36 belonging to Adenovirus subgroup D was first isolated by fecal sample of a girl suffering from enteritis (Dhurandhar, 2014). Dhurandhar *et al*. (2000) reported that Ad-36 increased visceral fat, total fat and body weight. Ad-36 enhanced lipid accumulation in 3T3-L1 cells in the presence of adipogenic-inducer such as dexamethasone and insulin (Rogers *et al*., 2008). Na *et al*. (2014) reported that the anti-obesity and anti-inflammation effects using mulberry extract on Ad-36 were evaluated in vivo.

D. racemosum is one of perennial herbs belonging to the Hamamelidaceae and is known as a medicinal plant. Ko *et al*. (2011) studied DPPH radical scavenging activities of the extract from *D. racemosum*. However, scientific data on biological and physiological effects of the extracts are insufficient. In this study, *D. racemosum* inhibited adipocyte differentiation through

suppression of regulator peroxisome proliferator-activated receptor-γ (PPAR γ) genes and adipocyte-specific genes such as adipocyte protein 2 (aP2), and suppressed the replication of Adenovirus 36 in the cells.

Taken together, this results revealed that the suppression of adipogenesis by *D. racemosum* was caused by high total phenolic contents, expression inhibition of PPARγ and aP2 gene and replication inhibition of Ad-36. Therefore, the extract of *D. racemosum* might be one candidate for development of anti-Ad-36 and anti-obesity drugs.

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국문초록

조록나무 추출물의 아데노바이러스 36 증식 및 지질 축적 억제능

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지도교수 장 경 수

비만은 전 세계적으로 중요한 질환으로 다양한 합병증을 유발하는 주요 위험 인자이다. 비만을 일으키는 많은 원인들 중 바이러스 감염에 대한 관심이 높아지고 있다. 아데노바이러스 36은 동물 및 사람에게 비만을 유발하는 것으로 알려져 있다. 비만의 예방 및 치료를 위해 많은 연구가 되고 있지만, 천연물질을 이용한 아데노바이러스 36 증식을 억제하는 비만치료제에 대한 연구는 아직 이루어져 있지 않다. 선행연구로 항산화 및 항콜레스테롤 효능을 가지는 조록나무, 참가시나무, 다정큼나무를 선별하였으며, 본 연구에서는 3가지 천연물질을 이용하여 항산화, 항콜레스테롤, 항지질 효과를 비교하고 아데노바이러스 36 증식 억제능을 규명하였다. 3가지 추출물 모두 5 mg/ml의 농도에서 80% 이상의 항산화 효과를 나타냈으며, 양성 대조군인 Quercetin과 유사한 효과를 보였다. 또한 항산화 물질인 총 페놀류화합물의 함량을 측정한 결과 각각 134.53 mg

GAE/g extract, 85.20 mg GAE/g extract, 65.20 mg GAE/g extract 으로 DPPH 라디칼 소거능 결과와 상관성을 나타내었다. 또한 조록나무 추출물의 50 ㎍/ml의 농도에서 3T3-L1 지방세포의 지질 축적을 억제하였고 지질 축적 주요 유전자인 PPAR-γ(Peroxisome proliferator-actived receptor-γ)와 adipocyte protein 2 (aP2)의 발현 억제와 아데노바이러스 36 증식을 억제 하였다. 따라서 조록나무 추출물을 비만 및 아데노바이러스 36 치료제로 개발 할 수 있는 후보 물질로 사료된다.

주제어 : 조록나무, 아데노바이러스 36, 항지질, 항산화, 항콜레스테롤