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Expression of preadipocyte genes in apical papilla cells after treatment with crude water extract of *Cuscuta japonica* Choisy

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ABSTRACT

Background: *Cuscuta japonica* Choisy (Japanese dodder) is a well-known traditional Chinese herbal medicine that has been used since ancient times as longevity and rejuvenation remedies, especially, in the southern part of China. The effects of this herb are widely known and can be applied for the treatment of a number of physiological diseases, but there is a lack of evidence describing its effects.

Objectives: This study focused on observing global change of gene expression after long-term treatment with high dose of crude water extract from *Cuscuta japonica* Choisy.

Materials and methods: In this research, dodder seeds were blended and boiled in distilled water before freeze drying to preserve as dried powder. UHPLC-QTOF-MS/MS was employed to test for important compounds in the extract of *Cuscuta japonica* Choisy. The extract at the concentration of 250 µg/mL was tested with apical papilla cells for 10 days to screen for change of gene expression by RNA sequencing before confirmation with real-time PCR.

Results: UHPLC-QTOF-MS/MS result found glycosides, phenolic acids and flavonoids as the main components in dodder seed water extract. Results from next-generation sequencing showed that dodder seed water extract significantly altered expression of 19 genes in apical papilla cells treated with the extract for 10 days (11 genes were increased while 8 genes were decreased). RT-PCR result of *CD36* and *SCARA5* genes showed correlated results with RNA sequencing.

Conclusion: The active compounds found in dodder seed water extract were phenolic acid, flavonoids, polysaccharide, lignan and volatile oil. The RNA sequencing studied in apical papilla cells showed that dodder seed water extract affected only a few genes that played roles in metabolism which correlated to the properties of this herb that was described as supplementary in metabolic diseases.

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Introduction

Cuscuta chinensis, *Cuscuta australis* and *Cuscuta japonica* Choisy were important species of *Cuscutae Semen* recorded in Chinese Pharmacopoeia.[1] The *Cuscuta chinensis* Lam., *Cuscuta japonica* Choisy, and *Cuscutae* Semen seeds are well-known traditional medicinal herbs, commonly used for improving reproductive system, supporting liver and kidney functions, reducing pain in joints and knees, and treating pharyngitis.[2] *Cuscuta* Semen is a crude drug prepared from the seeds of *Cuscuta chinensis* Lam., and used as a tonic liver and kidney. Nowadays, the seeds of *Cuscuta australis* and *Cuscuta japonica* Choisy are used as substitutes for *Cuscuta chinensis* , which is not readily available. *Cuscuta japonica* is a unique parasitic plant widely distributed in eastern Asian countries.[3] Extract of *Cuscuta japonica* Choisy is a well-known traditional Chinese herbal medicine that has been used since ancient times as longevity and rejuvenation remedies, especially, in the southern part of China. Effects of this herb are widely known and can be applied for the treatment of a number of physiological diseases, but there is a lack of evidence describing its effects. However, few evidences have been elucidated as mechanisms for the action of *Cuscuta japonica* Semen. The study by Moon *et al*. demonstrated that *Cuscuta japonica* Choisy had an enhancing effect on cognitive function and the histological mechanisms that support this improvement in cognitive function may be mediated by an increased level of neurogenesis in the mouse hippocampus.[4] Mushroom Tyrosinase inhibitory activity of *Cuscuta japonica* Choisy was presented by Suk *et al*.[5] The study of Jang et al indicated that aqueous fraction from *Cuscuta japonica* seeds inhibited p38 MAPK phosphorylation with suppressed cAMP levels and subsequently down-regulated MITF and TRP expression, which resulted in a marked reduction of melanin synthesis and tyrosinase activity in α-MSH-stimulated murine melanoma cells.[6] The content of polysaccharide in *Cuscuta japonica* was high, which has higher medical value.[7] *Cuscuta chinensis* polysaccharide showed benefits on glucose-lipid metabolism in diabetic rats induced by streptozotocin. *Cuscuta* chinesis plant was effective in reduced blood glucose of type 1 diabetes mellitus rats.[8] *Cuscuta chinensis* showed anti-obesity properties by modulating hepatic arginase and nitric oxide production and metabolic pathways related to hepatic triglyceride metabolism.[9]

This study presented the simple method for crude extraction by blending and boiling in water which still provided the active components such as phenolic acids and flavonoids that can be used for prevention of diseases. Most of the previous studies employed solvent extractions which were not common in daily life use. More over the effect of crude water extract on global gene expression in primary cell culture was performed.

Apical papilla cells were used as a model for study of gene expression in this study because they contained a population of mesenchymal stem cells[10] and other type of cells[11] which were easy to obtain from routine tooth extraction. Apical papilla cells could be used to test for global change of gene expression in according to the long-term treatment with crude water extract from *Cuscuta japonica* Choisy. In the parallel study, human gingival cells were treated with 250 µg/mL *Cuscutus japonica* Choisy water extract. The result from real-time PCR indicated that this amount clearly regulated *CD36* and *SCARA5* genes. We followed the previous treatment protocol to observe these 2 genes with apical papilla cells.

Materials and methods

Preparation of dodder seeds water extracts

Dodder seeds were obtained from local trader in Sichuan (Sichuan Shamgracy Pharmaceutical Co., LTD). Commercial *Cuscutae Semen* was *Cuscuta japonica* Choisy as indicated on the package (Figure 1). The voucher specimen of commercial *Cuscuta japonica* Choisy from Sichuan was previously identified by Prof. Yu-lin Lin (Institute of Medicinal Plant Development (IMPLAD), Chinese Academy of Medical Science and Peking Union Medical College) and deposited at the herbarium of IMPLAD, Chinese Academy of Medical Sciences and Peking Union Medical College. 1 Dried dodder seeds amount of 200 grams were blended in a blender for 2 minutes with 3,000 mL of distilled water before boiling for 5 minutes. After that, the boiled solution was cooled down at 4 °C overnight. The next day, the solution was filtered through white gauze. The filtrate was aliquoted in lyophilized flasks and frozen at -20 °C before lyophilized into powder for 7 days.

Figure 1. *Cuscutae Semen. Seeds of Cuscuta japonica Choisy bought from Sichuan Shamgracy Pharmaceutical Co., LTD.*

UHPLC-QTOF-MS/MS analyses

The UHPLC analyses were carried out on an AB Sciex ExionLC system (AB SCIEX, Foster City, CA, USA), equipped with an ExionLC Solvent Delivery System, an ExionLC AD Auto-sampler, an ExionLC AD Column oven, an ExionLC Degasser, an ExionLC AD Pump, an ExionLC PDA Detector, and an ExionLC Controller. Analytical column was a Shim-pack XR-ODSII column (2.0 mm i.d. × 75 mm). Column oven temperature was set at 30 °C. The mobile phases consisted of water containing 0.1% formic acid (solvent A) and acetonitrile (solvent B). The flow rate was set at 0.3 mL/min.

Binary gradient was applied with linear interpolation as follows: 18.00 min, 45.0% B; 23.00 min, 100.0% B; 24.00 min, 100.0% B; 24.01 min, 5.0% B; 27.00 min, 5.0% B; The UHPLC-QTOF-MS/MS detection was conducted on a Sciex QTOFTM X500R system with a TurboIonSpray source both in the positive and negative electrospray ion mode (AB SCIEX, Foster City, CA, USA). Parameters of the electrospray ionization applied in the positive mode are: ion spray voltage, 5500V; ion source temperature, 550 °C; curtain gas, 35 psi; Ion source gas 1 (GS 1), 55 psi; Ion source gas 2 (GS 2), 55 psi; and declustering potential (DP), 50V. The mass ranges were set at m/z 60-2000 Da for the TOF-MS scan and 50-2000 Da for the TOF MS/MS experiments. In IDA-MS/MS experiment, the collision energy (CE) was set at 35 eV, and the collision energy spread (CES) was 0 eV for the UHPLC-QTOF-MS/MS detection. Parameters of electrospray ionization applied in the negative mode are: ion spray voltage, -4500V; ion source temperature, 550 °C; curtain gas, 35 psi; Ion source gas 1 (GS 1), 55 psi; Ion source gas 2 (GS 2), 55 psi; and declustering potential (DP), -80V. The mass ranges were set at m/z 60-2000 Da for the TOF-MS scan and 50–2000 Da for the TOF MS/MS experiments. In the IDA-MS/MS experiment, the collision energy (CE) was set at -35 eV, and the collision energy spread (CES) was 0 eV for the UHPLC-QTOF-MS/MS detection. The most intensive 10 ions from each TOF-MS scan were selected as MS/MS fragmentation. Dynamic background subtraction (DBS) was applied to match the information dependent acquisition (IDA) tests for UHPLC-QTOF-MS/MS detection. The LC-MS/MS data was analyzed using PeakView® 1.2 software (AB SCIEX, Foster City, CA, USA).

Qualitative assay for glycosides by Molisch's test

Dried dodder seed water extract was dissolved in distilled water to the concentrations of 5000, 2500, 1250 and 625 µg/mL. Two mL of each concentration was prepared in the test tube and 2 drops of Molisch's reagent were added and mixed. One mL of conc. sulfuric acid was slowly overlayered without any mixing. The purple ring was observed in positive sample. Glucose diluted in distilled water at the concentrations 5000 µg/mL, 2500 µg/mL, and 1250 µg/mL were used as positive controls.

Apical papilla cell culture

Molar teeth were collected in routine tooth extraction according to a protocol approved by the Ethics Committee, Faculty of Dentistry, Chiang Mai University (68/2019). Apical papilla cells were obtained from apical papilla tissues of non-carious molars. The 18- to 25-year-old healthy patients were recruited (n=2). The apical papilla tissues were detached and digested by using 3 mg/mL collagenase I and 4 mg/mL dispase II for 45 minutes at 37 °C. Cells were cultured in complete α-Modified Eagle medium (α-MEM) at 37°C and 5% CO_2. Cells at the third passage with 80% confluent were used. Apical papilla cells were seeded into 6 well plates at the density of $1x10^5$ cells per well and maintained in 5% CO_2 at 37 °C for 24 hours to allow cell attachment. In the next day, medium was discarded and replaced with complete DMEM containing 250 µg/mL dodder extract. Apical papilla cells were seeded into 6 well plates at cell density of $1x10^5$ cells per well cultured in plain complete DMEM were used as control groups. Cultured media was changed every 3 days. Cells were treated for 10 days.

Cell staining

4% formaldehyde was added onto cell cultures and left for 10 minutes at room temperature. After that, 4% formaldehyde was discarded and replaced with methylene blue solution for 5 minutes. Methylene blue was washed out with tap water and cell morphology was observed under the inverted microscope.

RNA Sequencing (Transcriptomic assay)

The RNA sequencing was performed to observe Differential Gene Expression (DGE). Total RNA extraction was performed following the instruction from Nucleospin Kit. RNA samples were analyzed by Illumina platform, Novogene, Hong Kong. Differentially expressed genes between control sample and test sample at 10 days culture were analyzed by log2 fold change of Fragments Per Kilobase Million (FPKM) value of treated sample compared with FPKM value of control sample with adjusted p(padj)<0.05.

Real-time PCR

Total RNA amounts were measured with Nanodrop 2000. The reaction solutions were prepared with 6 µL of RNA, 4 µL of 5x RT Master Mix and 10 µL of DEPC water. The RNA was converted to cDNA in the thermocycler using the following program: 37 °C 15 minutes, 50 °C for 5 minutes, 98 °C for 5 minutes. cDNA was stored at -20 °C. NO-RT control was prepared with the same method using 5x RT buffer. Real-time PCR reaction were prepared with 8 µL RNA, 10 µL SYBR Green Mastermix and 2 µL primer (the final concentration of each the primer was 1.0 µM) in the total volume of 20 µL. The PCR program was set as following: (1) Pre-incubation at 95 °C for 2 minutes. (2) PCR consisted of denature at 95 °C, annealing at 60 ºC and extension at 72 °C. Real-time PCR was performed for 40 cycles. The reference gene; *GAPDH* was used for normalization. The sequences of primers used in this study were; *GAPDH*: (F) 5'-AAATCCCATCACCATCTTCCAGGAGC-3', (R) 5'-CATGGTTCACACCCATGACGAACA-3', *CD36*: (F) 5'-ATGTAACCCAGGACGCTGAG-3', (R) 5'-GTCGCAGTGACTTTCCCAAT-3', *SCARA5* (F) 5'-GGAACATCTCCCTCGCGAAA-3', (R) 5'-CTCGGTCACCTTTGAACCCA-3'. The experiment was divided into 2 groups: the control (cells cultured in complete DMEM) and the treatment (cells cultured in DMEM containing 250 µg/mL dodder seed extract). Duplicate pool samples of controls and tests were prepared. Fold change of gene expressions were presented in the form of mean±SD.

The relative quantification for the gene expression was normalized to *GAPDH* using Light Cycler®480 software release 1.5.0 SP4. This program used Advanced Relative Quantification which is a highly reliable results due to the extended range of parameters called the E-Method (Efficiency Method). The E-Method data are based on the true efficiency of each reaction and the final results are automatically calculated from the crossing point (Cp) values of the target (unknowns and calibrator) and the reference (unknowns and calibrator).

Results

Extraction

Dried powder of dodder seed water extract 14.97 g was obtained when 200 grams of dried dodder seeds were used in the extraction and %yield was equal to 7.5% (Figure 2).

Figure 2. *Dodder seed extract powder obtained after lyophilized.*

The composition of dodder seed extract by UHPLC-QTOF-MS/MS analysis

Mass spectrum of the active components was shown in Figure 3. The important chemical constituents in dodder seed extract were shown in Table 1 including phenolic acids, flavonoids, polysaccharides, lignan and volatile oil.

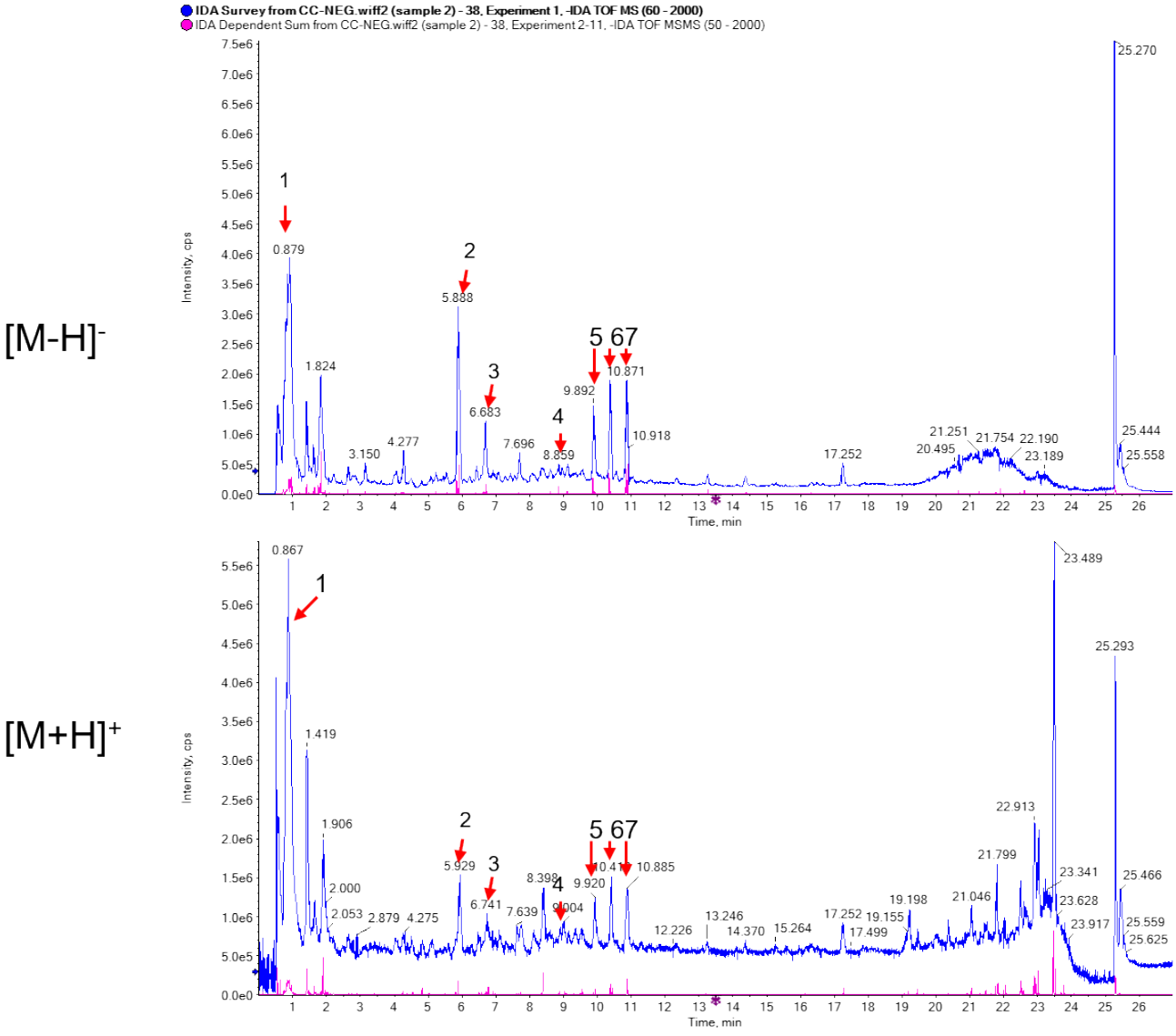

Figure 3. *Mass spectra of dodder seed water extract. The m/z of negative ion mode [M-H]- and positive mode [M+H]+ ions generated by electrospray were detected by positive and negative full scans.*

Table 1 Active compounds found in dodder seed extract.

Peak	Class	$[M-H]^-$ time	$[M-H]^-$ m/z	$[M+H]^+$ time	$[M+H]^+$ m/z	Name
1	Polysaccharides			0.73515	151.0256638	L-Arabinose
1	Lignans	0.8895	357.12945			Pinoresinol
2	Volatile oil	5.8217	137.034759	5.87393	139.0298671	2-Pentylfuran
2	Phenolic acid	5.8348	353.113443	5.88572	355.0795712	Chlorogenic acid
3	Phenolic acid	6.6975	179.047401	6.72505	181.0370603	Caffeic acid
4	Phenolic acid	8.603	163.051903	8.63547	165.0437828	P-coumaric acid
4	flavonoids			8.86682	303.0308298	Quercetin
4	flavonoids	8.8549	609.188342	8.87522	611.1220516	Rutin
4	flavonoids	8.7412	463.25218	8.89603	465.0731793	Hyperoside
5	flavonoids			9.92977	317.0441563	Isorhamnetin
5	Phenolic acid	9.8595	515.155518	9.94663	517.1002181	3,4-Dicaffeoylquinic acid
6	Phenolic acid	10.345	515.155256	10.354	517.1027363	3,5-Dicaffeoylquinic acid
7	Phenolic acid	10.836	515.155166	10.8821	517.1022455	4,5-Dicaffeoylquinic acid

Qualitative assay for glycosides by Molisch's test

Qualitative analysis of glycosides was confirmed using Molish's test. The result was shown in Figure 4. The observed purple rings in test tubes revealed that dodder seed extract contained glycosides.

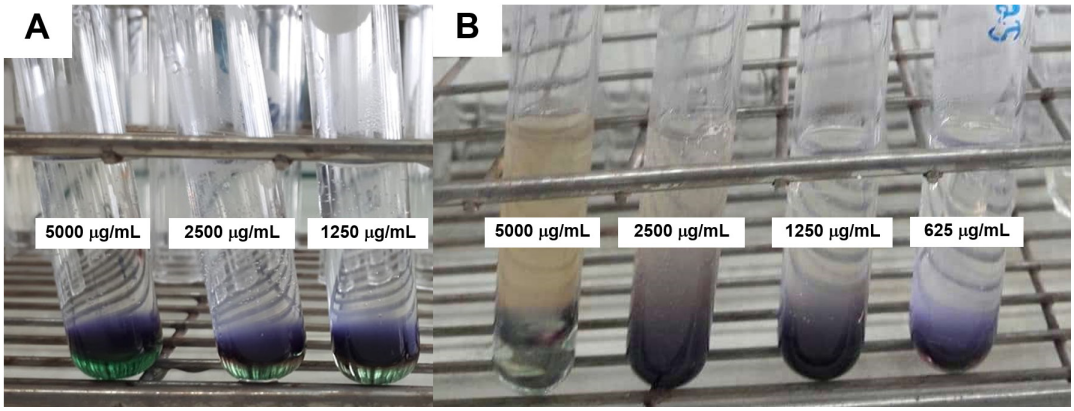

Figure 4. *(A) Formation of purple rings of glucose at concentrations of 5,000 2,500 and 1,250 µg/mL, which were used as positive control (B) Formation of purple rings of extracts at concentrations of 5,000 2,500 1,250 and 625 µg/mL respectively.*

Cell staining

Cell staining with methylene blue indicated that cells proliferated to form monolayer and the morphologies were not different between control and test as shown in Figure 5.

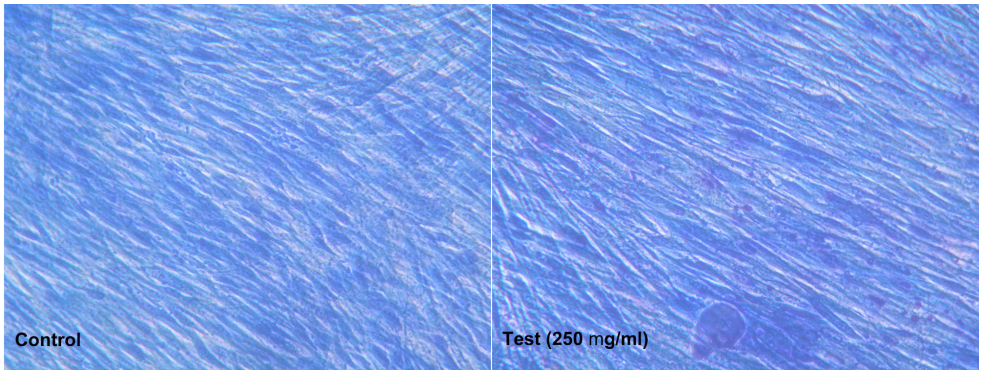

Figure 4. *Methylene blue staining of 10-day cultivation of apical papilla cells.*

Next-generation sequencing results in apical papilla cells

RNA quality control indicated that the RNA samples were passed with good purity and integrity. Next-generation sequencing identified more than 20,000 genes. However, when considering the adjusted *p*-value less than 0.05, only 19 genes were changed significantly. Differential gene expression was shown in Table 2.

Table 2 Differential gene expression.

Gene Name	*C. japonica* extract	Control	log2Fold Change	padj
SAMD11	0.041029	1.194022	-4.7066	0.02409
SCARA5	0.205143	4.55221	-4.4395	1.98E-06
WNT2	0.533371	4.55221	-3.0817	0.001073
RGS18	0.451314	3.320875	-2.8659	0.016795
DMKN	2.995085	13.47006	-2.1673	0.022233
CXCL14	5.046513	20.9327	-2.0513	0.02409
CHL1	3.856685	15.14916	-1.9724	0.041304
CXCL12	96.86843	352.2366	-1.8624	0.040733
C3	52.4345	13.76857	1.9288	0.033529
SFTPC	15.05748	3.880572	1.9548	0.045014
IL32	9.641712	2.462671	1.9669	0.065823
CTNND2	8.082627	2.014913	2.0015	0.063881
PIP	13.66251	3.246248	2.0717	0.030333
PLCXD3	12.0624	2.761177	2.1252	0.02409
SFTPB	6.56457	1.417901	2.2071	0.033529
KISS1	6.85177	0.93283	2.8703	0.000907
CD36	2.954056	0.373132	2.9686	0.011623
TM4SF4	0.8616	0	7.4706	0.040733
IGHA1	0.8616	0	7.4706	0.040733

Confirmation of *CD36* and *SCARA5* by real-time PCR

Apical papilla cells were treated with 250 µg/mL of dodder seed extract for 10 days. Replicate controls and tests were prepared. The expressions of both *CD36* and *SCARA5* were correlated with the result from Next-generation sequencing (Figure 6). *CD36* showed increased expression. *CD36* was a free fatty acid carrier.[12] It was possible that the increased expression of *CD36* was the effect of phytoestrogen because estrogen was reported to increase the expression of free fatty acid transporter (*CD36*) in the plasma membrane of the cardiomyocyte.[13] Scavenger receptor class A member 5 (*SCARA5*) showed increased expression. *SCARA5* also played a role as ferritin receptor.[14]

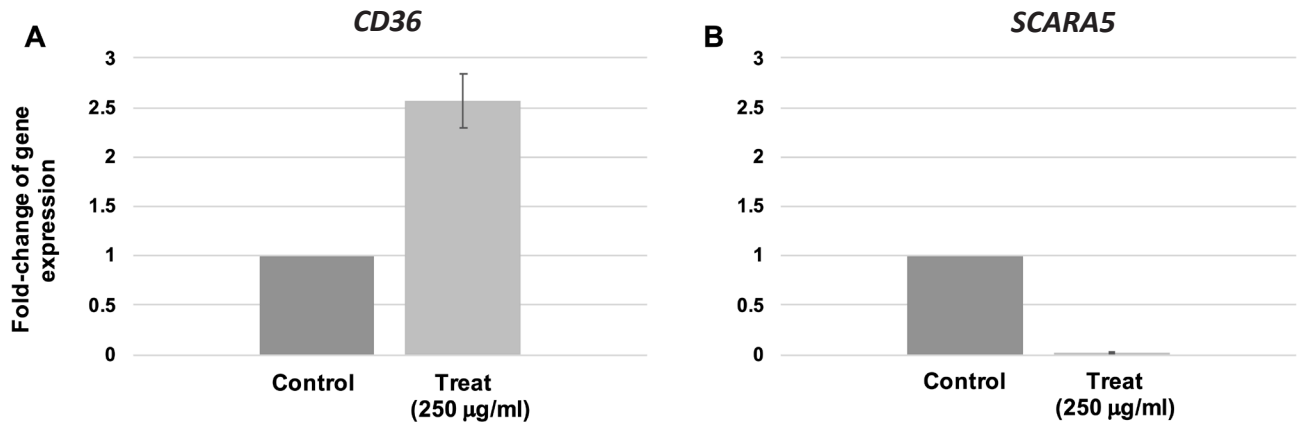

Figure 6. *(A) CD36 gene expression in duplicate experiments showed that the expression of CD36 increased by 2.6 times compared with the control group at 10 days. (B) SCSRA5 gene expression in duplicate experiments indicated that the expression of SCARA5 was dramatically reduced compared to the control group at 10 days. Real-time PCR results of CD36 and SCSRA5 gene expressions were correlated with the result from RNA sequencing.*

Discussion

The main substances found in dodder seed water extract were polysaccharides, phenolic acids and flavonoids. The phenolic acids found in dodder seed extract were chlorogenic acid, caffeic acid, p-coumaric acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid. In the previous studies, phenolic acids showed important roles in oxidative prevention and metabolism. 3,5-Dicaffeoylquinic acid was a potent α-glucosidase inhibitor which contributed to the antihyperglycemic action of the compound.[15] The beneficial effects of 3,5-dicaffeoylquinic acid support the traditional use of *Cuscuta japonica* Choisy for the treatment of diabetes.[16] 4,5-Dicaffeoylquinic acid maintained prostate cancer cell line in the S-phase.[17] 4,5-Dicaffeoylquinic acid was an inhibitor of pigmentation which could be developed as an active component for formulations to treat pigmentation disorders[18] and it might be concordant with the use as skin whitening product.

Chlorogenic acid possessed vital roles on regulation of glucose and lipid metabolic disorders, which are associated with the occurrence and progression of diabetes, obesity, hepatic steatosis, cardiovascular disease, and cancer.[19] Coumaric acid inhibited the differentiation of skeletal muscle cell and preadipocyte cells by inhibiting the genes that are responsible for myogenesis and adipogenesis.[20]

Quercetin, rutin, hyperoside and isorhamnetin were flavonoids found in dodder seed extract. These substances prevented cancer and oxidative stress. Isorhamnetin and quercetin were anti-acetylcholinesterase[21] while pinoresinol was a phytoestrogen with antitumor activity, inhibiting breast cancer cells.[22]

Studies in apical papilla cells (Table 2) showed that dodder seed extract clearly affected the expression of genes involved with metabolism and the transportation of substances across cell membrane. Some genes involved with signal transduction while some genes played roles as tumor promoter genes and tumor suppressor genes.

Increased expression of *CD36* indicated that cells were trying to uptake fatty acids into the cells because *CD36* controlled the transport of long-chain fatty acids into fatty acids-requiring organ including heart, skeletal muscle, and adipose tissue.[12, 23, 24] *CD36*-deficiency attenuates the development of high fat diet (HFD)-induced obesity and insulin-mediated inhibition of lipolysis was more potent in *CD36*(-/-) mice.[25] Upregulation of *CD36* in preadipocytes may contribute to the development of adipose tissue inflammation.[26] Elevated levels of *CD36* with a concomitant increase in adipocyte number.[27] *CD36* could promote adipogenesis in vitro as well as in vivo.[28] It seemed like the expression of *CD36* in this study indicated disadvantage of *Cuscuta japonica* water extract.

Significantly reduce expression of *SCARA5* was observed. Menssen et al. identified *SCARA5* as a new candidate gene possibly related to adipogenesis.[29] *SCARA5* was a positive regulator in fat tissue formation,[30] therefore, reduced expression of *SCARA5* should inhibit adipogenesis which concordant with the previous that coumaric acid inhibited adipogenesis. The concern of this study was that *SCARA5* was a tumor suppressor gene. The reduced expression may increase the risk of developing different types of cancer.[31] *SCARA5* could modulate the expression of epithelial-mesenchymal transition-related proteins.[32] Knockdown of *SCARA5* inhibits human aortic smooth muscle cells proliferation and migration through suppression of the PDGF signaling pathway. Thus, *SCARA5* may be a therapeutic target for preventing or treating vascular diseases involving vascular smooth muscle cell proliferation and migration.[33]

Both *CD36* and *SCARA5* were scavenger receptors.[34] *SCARA5* was a scavenger receptor class A found in epithelial, testis, heart and brain tissues as a receptor for ferritin. *SCARA5* bound to serum ferritin and stimulated endocytosis. *CD36* was a scavenger receptor class B which functioned as a receptor for thrombospondin-1, oxidized LDL, and long-chain fatty acid. Even though the expression of both *CD36* and *SCARA5* were ambiguous in this study, they indicated change in the regulation of lipid metabolism.

Change of other genes were found in differential gene expression but they were not confirmed by RT-PCR in this study. Upregulation of the *KISS-1* gene indicated that kisspeptin, encoded by the *KISS-1* gene may account for both reproductive and metabolic abnormalities reported in obese and diabetic rats.[35, 36] Kisspeptin-1 receptor involved in regulation of energy metabolism, obesity and adipocyte differentiation.[37] *PLCDXD3* gene correlated positively with insulin secretion and correlated negatively with HbA1c.[38] *TM4SF4* induced thiamine uptake which was important for cell survival because thiamine pyrophosphate played a vital role in metabolism and energy production.[39] The catenin-δ2 gene (*CTNND2*, encoding catenin-δ2) was associated with anxiety-disorder.[40] There was a report about using dodder seeds with other herbs to relieve anxiety. Additionally, many previous studies indicated that giant dodder (*Cuscuta reflexa*) could be promising for the development of phytomedicines for anxiety.[41] It was possible the related species like *Cuscuta japonica* Choisy might contain the same property on anxiety.

In addition, epithelial marker was also found upregulated. Dodder seed water extract increased the expression of *SFTPB* and *SFTPC* expression in the apical papilla cells. They were classified as differentiation markers of the alveolar and bronchiolar epithelial cells[42] which were lung-specific genes.[43] It was unclear whether differentiation of epithelial cells occurred or not which requires more detailed studies. Another important point was that some of the genes in Table 2 played roles as tumor promoter genes and tumor suppressor genes. Significant increase in *TM4SF4* was found in apical papilla cells treated with dodder seed extract. This gene was used as a prognostic marker for many types of cancer such as colon cancer,[44] stomach cancer[45] and lung cancer.[46]

At the same time reduced expression of immunoglobulin (*IGHA1*) was often found in metastatic or poor prognosis cancer.[47] In this experiment, the *IGHA1* gene showed a significantly increased expression. This gene was also used as a prognostic marker for cancer but as a preventive gene.[48] The increased expression of *IGHA1* causes tumor suppressor effect.[49]

Genes in some signaling pathways were changed. SMAD family was an intermediary in TGF-β signaling.[50] In

this experiment, *SMAD11* was reduced by 5 times in the group that was treated with dodder seed extract. Down-regulation of *WNT2* related to some signaling pathway and cancer. This gene produces cysteine-rich secreted glycoprotein, which acts as a paracrine[51] and autocrine.[52]

The important point of tumor promoter genes and tumor suppressor genes expression was the balanced effect between each other. Crude extracts contained several compounds and when total crude extract was applied to treat cells there could be a balance between expression of tumor promoter genes and tumor suppressor genes. Although more than 20,000 genes were identified but only 19 genes were changed significantly. It could be concluded that dodder seed water extract slightly altered transcriptome of apical papilla cells. Most of gene expressions indicated regulation of metabolism which concordant with the properties of this herb.

Conflicts of Interest

The authors declare no conflicting interests.

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