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Antioxidant activity of *Oenanthe stolonifera* D.C extract and *AMPK* activation on human liver cancer cells by anticancer effects

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Abstract

The aim of this study was to investigate the anticancer effect of *Oenanthe stolonifera* D.C extract (OJE) on Hep3B, a human liver cancer cell, and identify the anticancer mechanisms. When treated with 1.0 mg/mL of OJE in HEK-293 and HEP3B to test the anticancer effect based on cell viability and mobility, the extract showed no growth inhibitory effect on HEK-293, while Hep3B's cell viability and mobility significantly decreased to 62.5% and 48.6%, respectively. The RNA expression levels of AMP-activated protein kinase (*AMPK*), protein-53 (*p53*), and cyclooxygenase-2 (*COX-2*), which are key genes of carcinogenesis, were examined to investigate the mechanism of the anticancer effect. OJE downregulated *COX-2* and increased the expression of cancer suppressors *AMPK* and *p53* in a concentration-dependent manner. HPLC analysis was conducted to quantify secondary metabolites in OJE, and 0.50 mg/g DM of chlorogenic acid was identified as the main substance. Therefore, *Oenanthe stolonifera* D.C containing bioactive substances is valuable as a natural source for anticancer agent in the food and pharmaceutical industries.

Keywords: *Oenanthe stolonifera* D.C.; chlorogenic acid; Hep3B; *AMPK*; apoptosis; antioxidant; carcinogenesis; metastasis.

Practical Application: *Oenanthe stolonifera* D.C extract (OJE) has a high anticancer effect on human liver cancer cells by downregulating *COX-2* and increasing the expression of cancer suppressors *AMPK* and *p53*. The main substance in OJE is chlorogenic acid and it can be utilized as a natural anticancer agent in the food and pharmaceutical industries.

1 Introduction

Cancer has become the second most life-threatening disease after cardiovascular disease. The number of deaths caused by cancer is continuously increasing due to an aging population environmental pollution, and lifestyle changes like cigarette smoking, excessive alcohol consumption, poor diet, and lack of exercise (Moreno-Gómez et al., 2012; Sun et al., 2017). According to the World Health Organization, approximately 19,290,000 patients were diagnosed with cancer in 2021 (Kim, 2021; Misra et al., 2009). Among the various treatments administered against cancer, drug-based chemotherapy is the most widely used, and chemotherapy including first-generation chemical agents is actively implemented (Nowotnik & Cvitkovic, 2009). However, adverse effects, such as depression, drug resistance, and tumor recurrence, have been reported, shifting considerable focus toward developing natural anticancer medicines.

Mitochondria produce most ATP molecules through oxidative phosphorylation, which couples the transfer of electrons from nicotinamide adenine dinucleotide via the electron transport chain with the phosphorylation of ADP to ATP (Chen et al., 2004; Vasava & Mashiyava, 2016). However, some of the electrons designated for ATP generation can bypass the electron transport chain and directly reduce oxygen to very unstable forms, such as superoxide and superoxide anions. Such reactive oxygen species (ROS) oxidize proteins, lipids, nucleotides, and other macromolecules, irreversibly damaging DNA and cell (Misra et al., 2009; Wang et al., 2022). Organelles and constituting one of the causes of cancer, arteriosclerosis, heart disease, arthritis, and neurodegenerative disorders (Grigorov, 2012). As a part of their defense mechanisms, cells maintain homeostasis by scavenging ROS, converting superoxide into water and hydrogen peroxide with the help of antioxidant enzymes. However, the extent of ROS elimination using antioxidant enzymes, such as glutathione peroxidase, dismutase, and catalase (Ho et al., 1998; Orabi & Abou-Hussein, 2019). Alone is limited in an event of excessive ROS generation; thus, antioxidants that can be supplied as food have attracted considerable interest. Dietary micronutrients help the antioxidant defense system and several water-soluble substances, such as vitamin B and C, and lipid-soluble substances, such as vitamin A, D, and E, are known to have antioxidant properties (Skrivan et al., 2012; Costa et al., 2022). Also, antioxidants, such as polyphenols like quercetin, epigallocatechin, curcumin, resveratrol, and apigenin, play a promising role as efficient ROS scavengers, but their low activity and limited ability to penetrate both cellular and mitochondrial membranes highlight the need for developing new natural antioxidants that are more stable and effective than conventional antioxidants (Kim et al., 2006).

ROS also substantially influences the cancer microenvironment by initiating angiogenesis, metastasis, damages normal cells,

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and promoting proliferation (Malla et al., 2021). AMP-activated protein kinase (*AMPK*) is an essential cellular energy sensor that promotes ATP production by increasing the expression of proteins involved in catabolism and preserving ATP through the downregulation of biosynthetic pathways (Hardie, 2004). The tumor suppressor liver kinase B1 (*LKB1*) was discovered to be further upstream of *AMPK*, suggesting that its activity may be mediated via *AMPK*. This finding stoked interest in *AMPK* as a potential anticancer gene for cancer treatment (Cao et al., 2019). Excessive ROS accumulation affects many proteins involved in various regulatory pathways, such as *LKB1* and calcium/calmodulin-dependent protein kinase kinase 2 (*CamKK2*), which activates *AMPK*, resulting in adverse effects on cancer cell proliferation (Chaube et al., 2015; Herzig & Shaw, 2018). Cancer cells have a higher AMP/ATP ratio than normal cells because they convert glucose to lactate via working to produce ATP (Yu et al., 2017). As a result, *AMPK* gets activated and binds to protein-53 (*p53*), a cell cycle regulator, to arrest the cell in the G1 phase (the preparatory stage for mitosis) and to stop mitochondrial division, inducing cancer cell apoptosis (Høyer-Hansen & Jaattela, 2007; Li et al., 2004). On the other hand, cyclooxygenase-2 (*COX-2*), a cancer-promoting factor, converts arachidonic acid into prostaglandin E_2, which activates genes related to cancer cell growth and invasion, such as *COX-2* and vascular endothelial growth factor (*VEGF*), through the prostaglandin E_2 receptor. The activation of *AMPK* is reported to inhibit prostaglandin E_2 (Kim et al., 2012; Ching et al., 2020; Gam et al., 2021).

Recently, the United States National Cancer Institute recommended eating fruits, vegetables, whole grains, beans, and other plant foods as part of a cancer-preventing diet (Jannabi et al., 2020). Polyphenols like chlorophyll, resveratrol, and catechin, which are abundant in green and yellow vegetables, demonstrate anticancer activity by eliminating ROS, one of the most prominent carcinogens (Rajbhar et al., 2015; Chen et al., 2023). *Oenanthe stolonifera* D.C., a perennial plant belonging to the buttercup family, is known to contain kaempferol, quercetin and various types of vitamins, which exert liver protection and alcohol detoxification effects. It also possesses anti-inflammatory, antimutagenic properties and relieves hangovers (Jo et al., 2008; Park et al., 2014; Gam et al., 2022). Additionally, it is used to treat hypertension because it exhibits blood pressure-lowering effects. However, the anticancer mechanisms of antioxidants have not been studied adequately in the context of liver cancer.

The aim of this study was to demonstrate the anticancer potential of *Oenanthe stolonifera* D.C extract (OSE) against liver cancer and to validate its feasibility as a functional food and pharmaceutical substance. We extracted the bioactive substances in *Oenanthe stolonifera* D.C using ultrasound-assisted extraction (UAE) and identified the main compounds. The effect of OSE on the expression of *AMPK*, *p53*, and *COX-2* a key regulator of apoptosis in liver cancer cells, was evaluated to confirm its potential as a natural anticancer agent.

2 Materials and methods

2.1 Raw materials and reagents

The *Oenanthe stolonifera* D.C (MT622521) produced in the spring of 2021 was purchased from the Hanaro agricultural corporation (Pyeongtaek, Korea). Ethanol (99.5%) and distilled water (DW) were used as solvents for UAE. Gallic acid, quercetin, and ascorbic acid used as standards for the measurement of polyphenol, flavonoid, and antioxidant activity were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fetal bovine serum (FBS), Dulbecco's modified eagle medium (DMEM), and trypsin-EDTA used for cell cultured were purchased frsom Thermo Fisher (Waltham, MA, USA). Acetonitrile and acetic acid used in high performance liquid chromatography (HPLC) for quantitative and qualitative analysis were purchased from Sigma-Aldrich in HPLC grade.

2.2 Ultrasound-assisted extraction

Prior to the UAE, leaves, and stems of *Oenanthe stolonifera* D.C were dried in a dry oven at 60 °C for 24 hr, powdered using a food grinder (HMF-3000S, Hanil, Wonju, Korea), and passed through a 40-mesh sieve with particles smaller than 0.4 mm. Dried 1.0 g of *Oenanthe stolonifera* D.C was added to 10 mL of 50% ethanol and conducted extraction using an ultrasound device (SD-D250H, Sungdong Co., Hwaseong, Korea) at 60 °C for 30 min. After extraction, the supernatant was separated at 10,000 rpm for 10 min using a centrifuge (Lobogene 1236R, Gyrozen Co., Daejeon, Korea) and stored at -21 °C for a maximum of 3 month, for subsequent experiments.

2.3 Analysis of main substances using HPLC and HPLC-MS/MS

For quantitative and qualitative analysis of the main substances contained in OSE, the extract was filtered with a 0.45 μm PVDF syringe filter (Hyundai Micro Co., Seoul, Korea) and analyzed using HPLC (Agilent 1260 series, Agilent technology Inc., Santa Clara, CA, USA).

equipped with a Poroshell 120 EC-C18 column (4.6 × 150 mm, Agilent technology Inc., Santa Clara, CA, USA) and a diode array detector (DAD). The peaks of ingredients were separated by changing the mixing ratio of the mobile phase A (99% v/v acetonitrile/DW) and the mobile phase B (1% v/v acetic acid/DW), and then the separation was performed for 64 min at the column temperature of 30 °C (Table 1.). The flow rate was maintained at 0.5 mL/min and the substances in OSE were identified at an absorption spectrum between 190-640 nm using DAD. Qualitative analysis was performed by comparing the retention time and absorption spectrum of each separated substance. The quantitative analysis was conducted by comparing the peak area of the polyphenol standard.

The HPLC-MS/MS (Finnigan TSQ Quantum, Thermo Fisher (Waltham, MA, USA), which consisted of a binary pump, autosampler, vacuum degasser, RocTM C18 column (3.0 × 150 mm, Restek Ltd., Bellefonte, PA, USA). Extracts were then filtered with a 0.22 μm PVDF syringe filter and the peak was separated using mobile phase A (1% v/v formic acid/DW) and the mobile phase B (1% v/v formic acid/acetonitrile) (Table 1.). Chromatographic separation was operated at a column temperature of 28 °C, the flow rate of 0.2 mL/min, and injection volume of 10 μL under electrospray ionization (ESI)/turbo ion spray mode. Full scan data acquisition was performed by scanning from m/z 50 to 800 in negative ionization mode and was used for the mass spectrum and quantitative analysis.

2.4 Cell culture

Hep3B (human liver cancer cells) and HEK-293 (human kidney cells) were obtained from the Korean cell line bank (KCLB, Seoul, Korea) for the evaluation of OSE's anticancer activity. Cells were cultured in DMEM containing 10% FBS and 1% penicillin and cultured in an incubator (SA-MCO-18AIC, Panasonic, Osaka, Japan) at 37 °C with 5% CO_2. Unattached cells were washed with PBS and trypsin was used for cell collecting after 72 hr. The media was separated at 2,000 rpm for 2 min using a centrifuge and suctioned that media. Cell culture was performed using a cell culture flask with the inoculation of 5.0×10^4 cells/mL.

2.5 Cell cytotoxic measurement

The evaluation of cytotoxicity of OSE through comparison of cell growth was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2-5 diphenyl tetrazolium bromide) assay. Previously incubated 180 μL of 1.0×10^4 cells/mL was seeded into a 96 well culture plate and incubated for another 24 hr in a CO_2 incubator, and then treated with 20 μL of OSE (0.0-8.0 mg/mL). After 72 hr, culture media were removed and 100 μL of MTT reagent was added to each well and incubated for 4 hr at 37 °C. After removing MTT reagent, 100 μL of DMSO was added to the wells to dissolve the formazan crystals. Absorbance readings were performed at 450 nm using a microplate reader and the cell viability was calculated according to Equation 1.

$$\text{Cell viability}(\%) = \frac{\text{Abs (sample)}}{\text{Abs (control)}} \times 100 \quad (1)$$

2.6 Cells migration measurement

Previously incubated 1 mL of 1.0×10^5 cells/mL was seeded into a 24 well culture plate. After 24 hr of culture in CO_2 incubator, the center of each well was scratched using a 200 μL pipette tip to generate 0.9 mm of cell-free area, and various concentrations of OSE (0.0-1.0 mg/mL) were treated. After photographing the migration of cells using an optical microscope (Benulux B.V., Breda Co., Rotterdam, Netherlands), the migration distance of Hep3B was compared according to the following Equation 2 based on the wounded area of the control group.

$$\text{Cell migration}(\%) = \frac{\text{Scratch wounded (0hr)} - \text{Sample wounded}}{\text{Scratch wounded (0hr)}} \times 100 \quad (2)$$

2.7 Expression of cancer genes

To perform RT-PCR, Hep3B cells with an initial density of 1.0×10^5 cells/mL were seeded into 24 well culture plates and cultured in a CO_2 incubator for 24 hr. After collecting cells in a micro tube by centrifuging at 2,000 rpm for 2 min, the total RNA was extracted from cells with an AccuPrep® universal RNA extraction kit (Bioneer Co., Daejeon, Korea) and quantified using NanoDrop™ 2000 spectrophotometer (Thermo Fisher Sci., Inc. Waltham, MA, USA). RT-PCR was performed with a total 20 μL PCR tube containing 10 μL of premix (Genet bio, Daejeon, Korea), 1 μL of cDNA synthesis, and 9 μL of diethylpyrocarbonate (DEPC). The cDNA was amplified using *COX-2*, *p53*, and *AMPK* primers (Table 2.). Each PCR was electrophoresed on 1% agarose gel and visualized using the Gel Doc TM XR + system (Bio-Rad Co., Hercules, CA, USA) to compare the band intensity with the housekeeping gene (β-actin).

2.8 Statistical analysis

All the values were expressed as mean ± standard deviation (SD) and p of difference between groups were analyzed according to Student's t-test. The statistical analysis was carried out using Graphpad Prism software (Dotmatics, Boston, MA, USA). A statistically of differences between groups were considered significant at probabilities (p) < 0.05 levels.

3 Results

3.1 Quantification of main substance

We performed qualitative analyses based on retention time (RT) and diode-array detection (DAD) after high-performance liquid chromatography (HPLC) to identify the compounds that majorly influence the antioxidant activity. The main peak of OSE was found at 11.52 min, and the RT was consistent with that of chlorogenic acid. In the spectral analysis, the maximum absorption was found at 325 nm, and the spectrum was identical to that of chlorogenic acid. Thus, we concluded that the chief compound contributing to OSE's antioxidant activity is chlorogenic acid. The concentration of chlorogenic acid in OSE was estimated to be 0.50 mg/g DM using standards, which is approximately 4.5-fold higher than that in methanol-extracted *Oenanthe stolonifera* D.C, as reported by *Ryu et al.* chlorogenic acid was effectively extracted from the *Oenanthe stolonifera* D.C using ethanol (Ryu et al., 2020). *Ana et al.,* reported a chlorogenic acid

Table 1. HPLC and HPLC-MS/MS mobile phase compositions by gradient mode for the identification of main substances in OSE.

HPLC			HPLC-MS/MS		
Time (min)	A (%)	B (%)	Time (min)	A (%)	B (%)
0	100	0	0	95	5
5	85	15	11	0	100
50	50	50	14	0	100
60	0	100	15	95	5
64	100	0	20	95	5

Table 2. List of primers used to determine gene expressions of *p53*, *COX-2*, and *AMPK* using RT-PCR.

Primers	Forward (5'-3')	Reverse (5'-3')	Size (bp)
p53	CAGCCAAGTCTGTGACTTGCACGTAC	CTATGTCGAAAAGTGTTTCTGTCATC	292
COX-2	CGACTCCCTTGGGTGTCAA	GCTGGCCCTCGCTTATGAT	195
AMPK	CAACTGCAGAGAGCCATTCAC	GGTGAAACTGAAGACAATGTGCTT	76
β-actin	TCACCCACACTGTGCCCATCTACG	CAGCGGAACCGCTCATTGCCAATG	295

content of 0.11, 0.42, and 0.21 mg/g from *Malus sylvestris* leaves, *Crataegus pentagyna* fruits, and *Rubus fruitcosus* leaves extracts (Stoenescu et al., 2022). Therefore, we can conclude that the chlorogenic acid content of *Oenanthe stolonifera* D.C is higher than other natural products and it was effectively extracted by UAE. Chlorogenic acid exerts beneficial effects on various diseases, such as cancer, obesity, diabetes, cardiovascular, and neurodegenerative diseases. *In vivo* experiments have revealed that chlorogenic acid regulates lipid metabolism and reduces the production of free oxygen and peroxide, hence preventing diseases caused by oxidative stress in the cell (Preetha Rani et al., 2018; Zatorski et al., 2015).

Mass spectrometry (MS) analysis in the negative ion mode revealed that the m/z values of the main peaks of OSE were 190 and 353 (Figure 1). In this mode, the m/z value corresponds to the deprotonated molecule (Sim et al., 2008). An m/z of 353.2 was calculated to correspond to a deprotonated form of chlorogenic acid (MW = 354.3), reconfirming that the main substance detected in HPLC was chlorogenic acid. Since chlorogenic acid is an ester of caffeic and quinic acid, which functions as an intermediate in lignin biosynthesis, the m/z value of 191.6 was predicted as a molecular ion peak of quinic acid (MW = 192.2) in which caffeoyl was separated from chlorogenic acid. According to previous studies by *Rakesh et al.*, chlorogenic acid is generated when hydroxycinnamic acids, such as caffeine, are esterified with quinic acid, and of all the polyphenols, OSE mostly contains quinic acid and chlorogenic acid (Jaiswal et al., 2014). More in-depth studies on its functionality and mechanism at the level of gene expression are necessary in Hep3B cells.

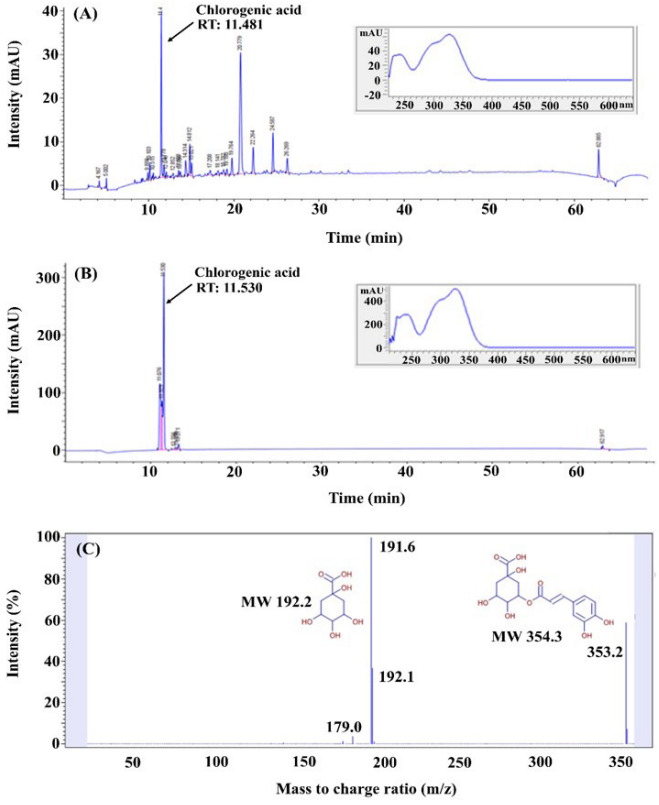

Figure 1. HPLC and HPLC-MS/MS analysis for identification and concentration measurement of main substances in *Oenanthe stolonifera* D.C extract (OSE). (A) Chromatogram and DAD spectrum (325 nm) of HPLC for qualitative and quantitative analysis of OSE, (B) Chromatogram and DAD spectrum (325 nm) of HPLC for chlorogenic acid standard, (C) HPLC-MS/MS spectrum of electrospray ionization for chlorogenic acid.

3.2 Effect of OSE on cell viability

To evaluate its anticancer activity, HEK-293 and Hep3B cells were treated with various concentrations of OSE and the corresponding cell viabilities were compared. HEK-293 is a cell line that was isolated from the kidney of a human and is popularly used in industrial biotechnology and toxicology research. The cell viability of HEK-293 was 98.4 ± 2.3% and 95.7 ± 1.4% when treated with 1.0 and 2.0 mg/mL of OSE, respectively, indicating that OSE did not inhibit the growth of HEK-293 at concentrations ≤ 1.0 mg/mL (Figure 2). The cell viabilities of Hep3B cells for the same OSE concentrations were 62.5 ± 3.2%, respectively, confirming that the growth of Hep3B was significantly inhibited at these doses ($p < 0.05$). This proves that OSE displays varied toxicity depending on the cell type: the cell viability of Hep3B decreased by three-fold compared with that of HEK-293. OSE is expected to exhibit anticancer activity without affecting normal cell growth at concentrations below 1.0 mg/mL.

3.3 Effect of OSE on cell migration

Cancer metastasis is the last stage of cancer development, wherein cancer cells migrate from the primary site to other organs through the bloodstream. More than 90% of cancer-related deaths occur at this stage, making it imperative to find active treatments to reduce metastasis (Ahn et al., 2008). For metastasis, cancer cells must migrate and invade through the extracellular matrix (ECM), intravasate into the blood circulation, attach to a distant site, and finally extravasate to form a distant focus (Han et al., 2003). To evaluate whether OSE inhibits metastasis, Hep3B migration was measured at the artificial cell free zone. The cell free zone significantly decreased as the concentration

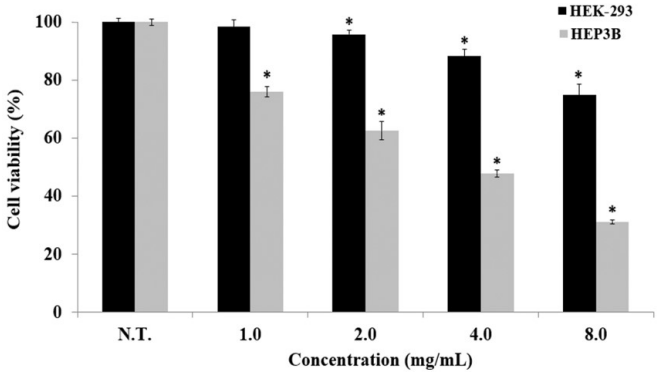

Figure 2. The cytotoxicity and anticancer activity of OSE on normal cell line HEK-293 and cancer cell line Hep3B. Significance levels (*$p < 0.05$) were evaluated through comparison with the control group (N.T.) by calculating the mean and standard deviation.

of OSE increased (Figure 3). When treated with 1.0 mg/mL of OSE, the migration of Hep3B was 48.6 ± 2% after 24 hr, a 1.7-fold suppression compared with that in the control group. After normalizing the cell free zone, we confirmed that 1.0 mg/mL of OSE suppressed cell migration compared with the control group after 24 hr. Therefore, OSE can potentially suppress cancer cell metastasis and proliferation, making it a promising natural anticancer agent.

3.4 Effect of OSE on cancer-related gene expression

Chlorogenic acid in *Corchorus olitorius* extract induces apoptosis by inhibiting cancer cell proliferation via *Bcl-2* inhibition and *Bax* upregulation, both of which are apoptosis-related genes (Tosoc et al., 2021). Similarly, OSE is also expected to reduce Hep3B proliferation by inducing apoptosis due to its high chlorogenic acid content. To determine whether OSE inhibited Hep3B growth via apoptosis, the cells were treated with 0.0-1.0 mg/mL of OSE, and the expression of apoptosis-associated genes, *AMPK*, *p53*, and *COX-2*, was examined using reverse transcription-polymerase chain reaction (RT-PCR). OSE treatment significantly affected gene expression in a concentration-dependent manner ($p = 0.0381$). *AMPK* and *p53* expression increased by 2.4- and 2.8-fold, respectively, compared with that in the control group, whereas *COX-2* expression decreased by 1.2-fold (Figure 4). The tumor suppressor *p53* is a transcription factor inducible by stress signals. Many studies have shown that the most obvious consequences of *p53* activation are cell cycle suppression and apoptosis. During apoptosis, when DNA damage, cancer gene

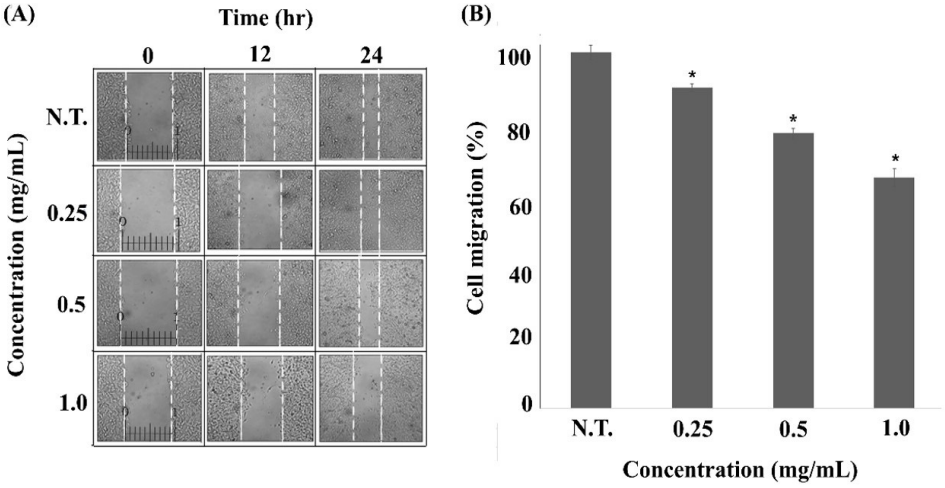

Figure 3. Comparison of migration in Hep3B by treatment of various concentrations of OSE. The experiment was conducted in triplicate, and the significance (*$p < 0.05$) was determined by calculating the mean and standard deviation, [and the non-treatment group was referred to as the control group (N.T.)] (A) Images showing the progress of wound closure on scratch migration Hep3B cells. (B) Graph showing the effect of OSE concentration on HEP3B migration at 24 hr.

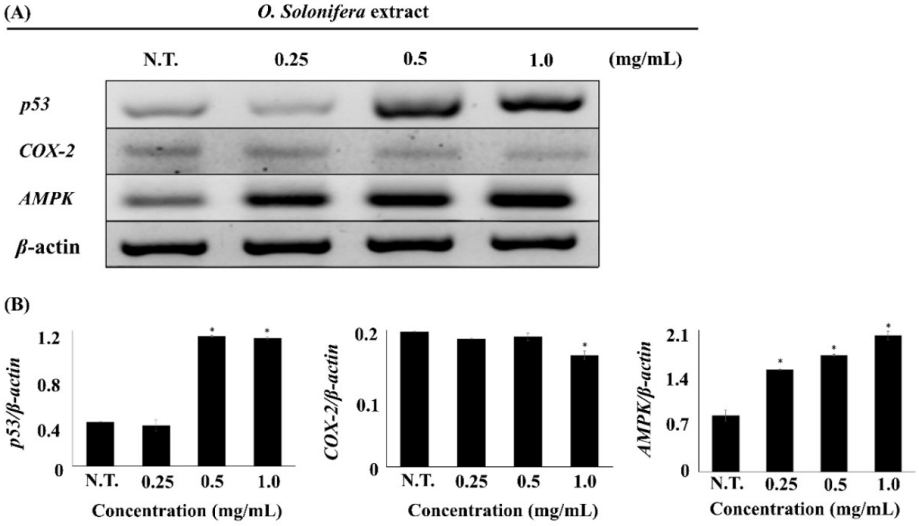

Figure 4. (A) Gel image showing the RT-PCR analysis from the expressions of *p53*, *COX-2*, and *AMPK* genes in Hep3B treated with OSE. (B) The intensity graph showing the RT-PCR from the expressions of *p53*, *COX-2*, and *AMPK* genes in Hep3B treated with OSE [and control group (N.T.).] The significance (*$p < 0.05$) was determined by calculating the mean and standard deviation.

activation, and nutrient deprivation occur, *p53* activates *p21*, consequently inhibiting the cell cycle or inducing apoptosis by binding to activated *AMPK* in the cytoplasm and transactivating apoptosis target genes, such as *COX-2, Bax* and *DR5* (Jung & Seo, 2006). Therefore, the correlation between OSE, *p53* upregulation, and *COX-2* downregulation demonstrates that OSE efficiently suppresses Hep3B growth and exerts an anticancer effect.

According to the findings of Park et al., the anticancer activity of black soybean (Glycine Max) extracts decreases *COX-2* expression and increases *p53* expression, triggering apoptosis and inhibiting the growth of HT-29 cells (Li et al., 2015; Park et al., 2015). This effect of the black soybean extract is likely due to increased apoptosis following the regulation of *p53* and *COX-2*. This study determined that OSE could arrest the cell cycle or inhibit Hep3B growth by promoting apoptosis through the regulation of *AMPK, p53*, and *COX-2* both of which are target genes for cancer treatment. As a result, we proved that *Oenanthe stolonifera* D.C can be potentially used as an ingredient of functional foods and naturally derived anticancer agents.

4 Conclusions

In this study, the composition of bioactive substances in OSE and its antioxidant activity and cytotoxic effects on Hep3B cells were investigated to confirm its anticancer effects. The main polyphenols were identified using HPLC and HPLC/MS/MS, and the anticancer properties of the extract were determined by evaluating the expression of cancer-related genes in OSE-treated Hep3B cells. HPLC and HPLC/MS/MS analysis revealed that chlorogenic acid is the main component contributing to the antioxidant activity of OSE. Cytotoxicity analysis showed that the growth of Hep3B cells was suppressed as the concentration of the extract increased, while the growth of HEK-293 cells was unaffected below a specific concentration. Therefore, we can conclude that OSE is a possible anticancer agent since it selectively inhibits the growth of cancer cells without inhibiting the growth of normal cells at concentrations ≤ 1.0 mg/mL. Moreover, the migration of OSE-treated Hep3B cells was significantly lower than that of untreated cells, suggesting the potential of the extract to suppress Hep3B invasion and metastasis. These effects of OSE are considered to be the result of apoptosis induction via the downregulation of *COX-2* and the upregulation of *AMPK*, *p53*. Hence, *Oenanthe stolonifera* D.C is a suitable natural ingredient for high-value-added foods and medicines due to its protective effects against liver cancer, high human safety, and easy availability of raw materials.

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