Biocatalysis and Agricultural Biotechnology 18 (2019) 101040

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

In vitro assessment of three different *artemisia* species for their antioxidant and anti-fibrotic activity

Arthanari Saravanakumar[a,b,*], Parthiban Periyasamy[a], Hyun Tae Jang[b,**]

[a] Vellalar College of Pharmacy, Maruthi Nagar, Thindal, Erode, 638012, Tamilnadu, India
[b] Department of Chemical Engineering, Hanseo University, 360 Daegok-ri, Seosan-si, 356 706, Chungcheongnam-do, South Korea

ARTICLE INFO

Keywords:
Artemisia
Extraxtion
Antioxidants
Anti-fibrotic

ABSTRACT

The aqueous extracts of three different *Artemisia* species (*Artemisia capillaris*, *Artemisia iwayomogi*, & *Artemisia annua*) were evaluated for their antioxidant potential and antiproliferation, antifibrotic effect using activated rat HSC-T6 cells. The antioxidant activities of these plant extracts were assessed by different *in vitro* methods, such as free radical scavenging assays by DPPH, Superoxide, Hydroxyl, Nitric oxide and H_2O_2, and Ferric reducing antioxidant power (FRAP), total antioxidant abilities, and Iron chelating activity. The total phenolic and flavonoid contents were examined as well. Antifibrotic effects were investigated for three *Artemisia* species by measuring the hydroxyproline content and analyzing the gene expression of the two main fibrogenic cytokines of TGF-β and α-SMA in activated HSC-T6 cells. Among the tested Artemisia species, *Artemisia capillaries* is highest in TPC, TFC, TAC and FRAP while compared to others with 900.91 ± 22.81 (μg/g of GAE), 2918.83 ± 109.37 (μg/g of QE), 43.21 ± 0.28 (μg/g of GAE) and 3880.24 ± 340.37 (μg/g of $FeSO_4$) respectively. The aqueous extracts of all *Artemisia* species reduced the proliferation of activated HSC – T6 cells. Regarding antifibrotic effect, the hydroxyproline content was significantly reduced in a concentration-dependent manner while treated with extracts. Further the activated HSCs drastically upregulated the fibrogenic cytokines (TGF-β, α-SMA), while the addition of three *Artemisia* extracts (50 μg/mL) significantly reduced these alterations. However, *Artemisia capillaris* and *Artemisia iwayomogi* enormously reduced both TGF-β and α-SMA expressions.

1. Introduction

Reactive oxygen species (ROS) and reactive nitrogen species (RNS), including free radicals, play a vital role in oxidation, which may oxidize proteins, lipids, or DNA in tissues, leading to degenerative/chronic diseases including cancer, diabetes, cardiovascular diseases, etc. (Lee et al., 2004; Mantena et al., 2008). The main role of antioxidants is that they can protect biological systems against oxidation or inhibit or delay the oxidation process (Sikorski, 2001). Commercially available synthetic antioxidants, such as BHA, BHT, and TBHQ, as well as their applications, are constrained for use in foods because they are suspected to be carcinogenic (Valentao et al., 2002). Plant-derived products offer a wide range of natural antioxidants which are considered to be superior to synthetic antioxidants.

Liver fibrosis is a very common disease that can result from many forms of chronic liver injuries, including alcoholism, persistent viral and helminthic infections, toxins, and hereditary metal overload (Bataller and Brenner, 2005; Friedman, 2008a). The activation of hepatic stellate cells (HSCs) plays a crucial role in the development of liver fibrosis. The formation of myofibroblast-like phenotype, extracellular matrix (ECM) proteins, and increased cell proliferation are the main events involved in the pathogenesis of liver fibrosis (Wu and Zern, 2000). During liver fibrosis, HSCs are activated by inflammatory cytokines as well as certain growth factors in paracrine and autocrine manners. Therefore, the suppression of HSC activation and proliferation, along with the induction of apoptosis in activated HSC, have been offered as therapeutic approaches for the treatment and prevention of hepatic fibrosis (Bataller and Brenner, 2005; Henderson and Iredale, 2007; Friedman, 2008b).

In Korea, a total of 28 species are present in genera of *Artemisia*, which belongs to the class Campanulales and the family Compositae (Lee, 1996). *Artemisia* herbs have long been widely used in foods and traditional medicine for the treatment of diabetes, hyperlipidemia, and hepatitis (Noori and Dawood, 1986; Nam et al., 1998; William, 2008). Based on their high consumption in the traditional market, we selected three genera of *Artemisia* plants for our study.

Artemisia capillaris (AC) Thunberg has long been widely used as traditional medicine in Asian countries, particularly to treat liver disorders such as hepatitis, jaundice, and fatty liver (Jang, 1975). AC and

* Corresponding author. Vellalar College of Pharmacy, Maruthi Nagar, Thindal, Erode, 638012, Tamilnadu, India.
** Corresponding author.
E-mail addresses: drsaravanakumara@gmail.com (A. Saravanakumar), htjang@hanseo.ac.kr (H.T. Jang).

https://doi.org/10.1016/j.bcab.2019.101040
Received 7 September 2018; Received in revised form 7 February 2019; Accepted 10 February 2019
Available online 14 February 2019
1878-8181/

Abbreviations used

TPC	Total phenolic contents
TFC	Total flavonoids content
TAC	Total antioxidant capacity
GAE	Gallic acid equivalent
QE	Quercetin equivalent
FRAP	Ferric reducing antioxidant power
$FeSO_4$	Ferrous sulphate
ROS	Reactive oxygen species
RNS	reactive nitrogen species
BHA	butylated hydroxy anisole
BHT	butylated hydroxy toluene
TBHQ	*tert*-butyl hydroquinone

its active constituents, including phenolic acids, coumarins, flavonoids, and 4-hydroxyacetophenone, have exhibited pharmacological actions against viral infections, oxidative stress, and obesity (Seo et al., 2010; Hong and Lee, 2009; Hong et al., 2009). In particular, AC has reported significant hepatoprotection in drug- or chemical-induced liver injury rodent models (Lee et al., 2007; Wang et al., 2012).

Artemisia iwayomogi (AI) Kitamura is a widely used valuable herb that is distributed throughout Korea and China (Jang, 1975). The pharmacological activities associated with AI and its key active compound, scopoletin, have previously been reported to show anti-inflammatory (Shin et al., 2006), antimicrobial (Seo et al., 2010), antioxidant (Seo and Yun, 2008), and hepatoprotective activities (Choi et al., 2005; Wang et al., 2012). In addition, several active components of AI such as chlorogenic acid, genkwanin 3-*O*-methyl isosecotanapartholide, and isosecotanapartholide have shown peroxy nitrite scavenging as well as the inhibition of nitric oxide (NO) production in LPS-activated macrophages (Ryu et al., 2003).

Artemisia annua L. (AA) has been used globally to treat various disorders, particularly malaria fever, across all ages (Hsu, 2006; Wright et al., 2010). The active compound is artemisinin, a sesquiterpenoid approved by the World Health organization (WHO) for the treatment of drug resistant and cerebral malaria (Bhattarai et al., 2007).

The objectives of this investigation are to assess the *in vitro* free radical scavenging (DPPH, H_2O_2, Hydroxyl, Nitric oxide, and Superoxide), total antioxidant abilities, Fe^{2+} metal chelating activity, Ferric reducing antioxidant power (FRAP), total phenol, flavonoid contents and antiproliferation, anti-fibrotic effect of three *Artemisia* species using activated HSCs. The results of this study will provide a better understanding of the scavenging properties of these plants and allow for the identification of high value plants with prominent antioxidant activity for further investigation.

2. Materials and methods

2.1. Chemicals and reagents

Aluminium chloride ($AlCl_3$), Catechin, and Gallic acid were purchased from Acros Organics. 2,4,6 – tripyridyl - s-triazine (TPTZ), 1, 1-diphenyl, 2-picryl hydrazyl (DPPH), Ascorbic acid, 2-2 azino-bis (3ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), PBS buffer, Folin–Ciocalteu's phenol reagent, Gallic acid, Quercetin, Methanol, Nitro blue tetrazolium (NBT), Ferrous sulphate, Sodium hydroxide (NaOH), Dimethyl sulphoxide (DMSO), Salicylic acid, Hydrogen peroxide, Sodium nitroprusside, Sulphanilamide, Phosphoric acid, N− (1- naphthyl) ethylenediamine dihydrochloride, EDTA, Sodium carbonate (Na_2CO_3) and, Sodium nitrite ($NaNO_2$) were purchased from Sigma Chemical Company. All other chemicals and reagents used were of analytical grade.

2.2. Plant materials and extraction

Three *Artemisia* medicinal herbs were collected from Jeong – Seoung Oriental medicine market, Daejeon, South Korea and was authenticated by a professor in College of Pharmacy Kangwon National University, South Korea. Herbs (each 100 g) were mixed and boiled in 1 L of distilled water for 150 min using an automatic non-pressure pot (Daewoong, Seoul, Korea). The suspension was filtered using a 300-mesh filter paper (50 mm) (Advantec, Toyo Roshi Kaisah, Tokyo, Japan), and the filtrate was concentrated in a rotary evaporator and lyophilized at – 80 °C for 48 h. The extraction yields were 5.63% (AC), 8.91% (AI), and 8.63% (AA), and each extraction was stored at – 80 °C until further use.

2.3. Total phenolic content

TPC of aqueous extracts were determined by employing Folin–Ciocalteu reagent (Kim et al., 2003). First, 400 µL of Folin ciocalteu reagent was mixed with 100 µL of aqueous extracts (0.2–10 mg/mL) of the three *Artemisia* species in a volumetric flask. The solution was placed at 25 °C for 5–10 min and mixed with 0.5 mL of Na_2CO_3 (1 M) solution, then the mixture was finally diluted to 10 mL in a volumetric flask with deionized distilled water. Prior to taking the absorbance at 765 nm, the mixture was placed for 2 h at 25 °C. A calibration curve was plotted for gallic acid and the total phenolic contents were calculated as per µg gallic acid (GAE) equivalents per gram of dried fraction (µg/g).

2.4. Total flavonoid content

A calorimetric assay was used to estimate the total flavonoid content. In a volumetric flask of 10 mL, an aliquot of 0.1 mL of aqueous extracts (0.1–10 mg/mL), distilled water (4.0 mL), 5% $NaNO_2$ (0.3 mL), and 10% $AlCl_3.6H_2O$ (0.3 mL) was mixed and placed in room temperature for 6 min. Then, 2 mL of NaOH (1 M) was added, and the solution was diluted with 2.4 mL of distilled water. The absorbance of the colored solution was recorded at 510 nm against a blank (containing all reagents except sample) in UV spectrophotometer (Molecular devices). Quercetin was used to plot the calibration curve. The total flavonoid content was calculated as µg Quercetin equivalents per gram of dried extract (µg/g).

2.5. Antioxidant assays

2.5.1. DPPH assay

The scavenging activity of DPPH was assessed by the scavenging of 2, 2-diphenyl-1-picrylhydrazyl radicals (W. Brand-Williams et al., 1995). A stock solution of 0.3 mM DPPH was prepared by dissolving the DPPH in methanol. DPPH solution of 100 µL was added to 100 µL of three aqueous extracts at varying concentrations (0.01–0.5 mg/mL), then shaken vigorously. The absorbance was recorded at 517 nm after 15 min of incubation in room temperature. Water and DPPH was used as control. The experiment was conducted three times and averaged. The DPPH scavenging activity of various extracts was calculated by the following equation (eq-1).

$$\text{Percentage inhibition (\%)} = [(\text{Control Abs} - \text{Sample Abs}) / (\text{Control Abs})] \text{ X } 100 \quad (1)$$

2.5.2. Superoxide scavenging assay

The Alkaline DMSO method was used to determine the superoxide radical scavenging assay (Elizabeth and Rao, 1990). First, 10 µL of nitro blue tetrazolium (NBT) (1 mg/mL prepared in DMSO) was added into a microtiter plate, and 30 µL of different concentrations of extracts (0.2–10 mg/mL) or standard were introduced in to the wells. Then, 100 µL of alkaline DMSO (90 µL of DMSO + 10 µL of 5 mM NaOH) was added. The absorbance was measured at 560 nm in a

spectrophotometer (Molecular devices). DMSO was used as a control instead of sample. The percentage scavenging activities of the various extracts were calculated by the following equation (eq-2)

Percentage inhibition (%) = [(Test Abs – Control Abs) / (Test Abs)] X 100 (2)

2.5.3. Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was analyzed as described by Smirnoff and Cumbes (1989), with some minor modifications. To 1 mL of different concentrations of extract solution (0.2–10 mg/mL), 300 µL of $FeSO_4$ (8 mM) solution and 250 µL of H_2O_2 (20 mM) were mixed. In order to initiate the reaction, 250 µL of salicylic acid in ethanol (3 mM) was added. The reaction mixture was allowed to stand for 30 min in a water bath at 37 °C, after which, 450 µL of distilled water was added, and the mixture was centrifuged at 10000 rpm for 10 min. The absorbance of the supernatant was measured at 510 nm. Extracting solvent was used as control instead of sample. The percentage scavenging activities of the various extracts were calculated by the following equation (eq-3).

Percentage inhibition (%) = [(Control Abs – Sample Abs) / (Control Abs)] X 100 (3)

2.5.4. Nitric oxide scavenging assay

The nitric oxide scavenging capacity was measured by the method described by Sreejayan and Rao (1997), with some minor modifications. First, 500 µL of sodium nitroprusside (10 mM) was incubated with 500 µL of phosphate buffer (1 mM) and 30 µL of different concentrations of aqueous extracts (0.1–5 mg/mL) at 25 °C for 90 min. Following incubation, an aliquot of 1 mL solution (1:1) was prepared by mixing incubated solution with freshly prepared Griess reagent (2% of sulphanilamide in 4% phosphoric acid and 0.2% of N— (1- naphthyl) ethylenediamine dihydrochloride), and the absorbance was measured at 546 nm after 3–5 min. The percentage scavenging activities of the various extracts were calculated by the following equation

Percentage inhibition (%) = [(Control Abs – Sample Abs) / (Control Abs)] X 100 (4)

2.5.5. Hydrogen peroxide scavenging assay

Hydrogen peroxide scavenging assay was carried out according to the method described by Muller (1985), with some modifications. First, 80 µL of different concentrations (0.01–0.2 mg/mL) of aqueous extracts were mixed with 20 µL of H_2O_2 (10 mM) in a microtiter plate. Then, 100 µL of phosphate buffer pH 5 (0.1 M) was introduced in to the wells. Next, the plate was incubated at 37 °C for 5 min. Finally, 60 µL of ABTS (1.25 mM) prepared with 1 IU/mL of peroxidase was mixed, and the plates were kept for incubation at 37 °C for 10 min. The absorbance was measured at 405 nm. The percentage scavenging activities of the various extracts were calculated by the following equation (eq-4).

Percentage inhibition (%) = [(Control Abs – Sample Abs) / (Control Abs)] X 100] (5)

2.5.6. Ferric reducing antioxidant power (FRAP) assay

In order to evaluate the reducing power of extracts, the ferric ion reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996), modified for 96 well microplates, was used. FRAP reagent was prepared by mixing 10 mM/L of TPTZ in 40 mM/L HCl, 0.02 M/L $FeCl_3$, and acetate buffer pH 3.6 in a 1:1:10 ratio, respectively. Following the addition of different concentrations of extract or standard (10 µL) to 290 µL of FRAP reagent (substituted with distilled water in blank probe), the absorbance at 593 nm was measured after 6 min. All samples were prepared in triplicate, and the mean values of reducing power were expressed as µg of ferrous sulphate equivalent per g of dry weight (dw), calculated according to regression equation (Y = mx + C regression line) derived from the standard calibration curve.

2.5.7. Total antioxidant capacity (TAC)

The total antioxidant capacity was measured by taking 90 µL of phosphate buffer (1 M, pH 7.2) was mixed with 50 µL of myoglobin (18 µM), 20 µL of ABTS (3 mM), and 20 µL of different concentrations of sample (0.01–0.2 mg/mL) or standard. After 3 min incubation in room temperature, 20 µL of H_2O_2 (250 µM) was added and again incubated in room temperature for 5 min. The absorbance was then measured at 600 nm. In order to obtain good results, the whole experiment was carried out in cold condition. Gallic acid was used as a standard, and the results were expressed as µg gallic acid (GAE) equivalents per gram (GAE/g) of dried extract (µg/g) on the basis of regression equation (Y = mx + C regression line) using the standard plot.

2.5.8. Iron chelating assay

The ability of extracts to chelate Fe^{2+} was determined as previously described (Dinis et al., 1994), with some minor modifications. Briefly, 0.1 mL of $FeSO_4$ (0.1 mM) was mixed with 0.1 mL of different concentrations of extracts (0.1–5 mg/mL) or standard and 0.1 mL of Ferrozinc (0.25 mM) in microtiter plates. This was then allowed to stand in room temperature for 10 min. Next, the absorbance was measured at 562 nm. EDTA was used as a standard. The percentage chelating activities of various extracts were calculated by the following equation

Percentage inhibition (%) = [(Control Abs – Sample Abs) / (Control Abs)] X 100 (6)

2.6. Anti fibrotic activity

2.6.1. Hepatic stellate cells (HSC-T6)

Hepatic stellate T6 (HSC-T6) cells, an immortalized rat hepatic stellate cell line, were kindly provided by Prof. SL Friedman, Mount Sinai School of Medicine, New York. HSC— T6 cells were cultured in DMEM supplemented with 10% heat-inactivated fetal bovine serum, 100 IU/mL penicillin, and 100 mg/mL streptomycin at 37 °C in a humidified atmosphere of 95% air – 5% CO_2.

2.6.2. Anti proliferation assay

HSC-T6 cells (3×10^3 cells per well) (180 µL) were cultured in 96 well plates for 24 h. Different concentrations of herbal extracts (5, 10, 50, 100 µg/mL) were added to the cells and further incubated for 24 h. Finally, 20 µL of CCK-8 kit (LPS solutions) solution was added in order to measure the cell proliferation after 1–3 h incubation at 37 °C in 5% CO_2. The absorbance was measured by an enzyme linked immune sorbent assay (ELISA) reader (Versamax tunable microplate reader, Sunnyvale, California, USA) at a test wavelength of 450 nm and a reference wavelength of 600 nm.

2.6.3. Hydroxyproline content

Collagen was determined by estimating the hydroxyproline content, which is an amino acid characteristic of collagen. HSC-T6 cells were lysed after treatment with different concentrations of the three different *Artemisia* species (10, 50, 100 µg/mL) for 48 h. The lysates were hydrolyzed in 2 mL of 6 N HCl for 24 h at 110 °C, and evaporated to dryness in order to remove the acid. 100 µL of 0.05 M $CuSO_4$, 250 µL of 2.5 N NaOH, and 250 µL of 7% chloramine T were mixed with the residue. The solution was kept in room temperature for 5 min, then heated at 70 °C for 10 min in a water bath, followed by cooling in ice for 5–10 min. Then, 200 µL of 8 N H_2SO_4 and 1.25 mL of 5% *p*-dimethylamino benzaldehyde in isopropanol (Ehrlich's reagent) were mixed, then again heated at 70 °C for 40 ± 5 min in a water bath. *p*-dimethylamino benzaldehyde in isopropanol was prepared by heating the solution in a water bath at 65–70 °C for 10 min. The absorbance was measured at

558 nm using a UV spectrophotometer. Hydroxyproline was used to prepare a standard curve. The values obtained were expressed as μg of hydroxyproline.

2.6.4. Gene expression analysis by qRT-PCR

HSC-T6 cells were maintained in Dulbecco's modified Eagle's medium with 10% fetal bovine serum and incubated at 37 °C in a 5% CO_2 humidified atmosphere. The cells were treated with 50 μg/mL of each of the three *Artemisia* aqueous extracts. Total RNA was extracted after 24 h treatment with plant extracts using TRIzol reagent (Molecular Research Center, Cincinnati, OH, USA). The harvested cells after 6 h of incubation were used to isolate the mRNA. The total RNA was measured by NanoDrop 2000 (Thermo Scientific). The extracted RNA concentration was adjusted to be 2 μg per reverse transcription reaction using a High-Capacity cDNA Reverse Transcription kit (Ambion). The primer sequences used were (forward and reverse, respectively) β-actin (CTAAGGCCAACC GTGAAAAGAT and GACCAGAGGCATACAGGGACAA), α-SMA (AACACG GCATCATCACCAACT and TTTCTCCCGGTTGGCCTTA), and TGF – β (AGGAGACGGAATACAGGGCTTT and AGCAGGAAGGGTCGGTTCAT), respectively. Reactions were performed with 8 μL of iQ SYBR Green Supermix, 1 μL of 10 pmol/L primer pairs, 8 μL of distilled water, and 3 μL of cDNA. Each PCR reaction was performed under the following conditions: 95 °C for 5 min, followed by 40 cycles of 95 °C for 1 min, 58 °C for 40 s, and 72 °C for 40 s, followed by a single fluorescence measurement.

2.7. Statistical analysis

All of the experiments were carried out in triplicate. The results were expressed as means ± standard deviations (SD). The analysis of variance and significant differences were examined by one way ANOVA followed by the Bonferroni *post hoc* test using SPSS software (version 13.0 for Windows, SPSS Inc., Chicago, IL). In addition, Pearson correlation coefficients were calculated between total phenolics, flavonoids, and antioxidant methods. In all methods, $p < 0.05$ was regarded as significant.

3. Results

3.1. Total phenolics and flavonoids content

Phytochemicals such as phenolic compounds are accepted as valuable in human health for maintaining and repairing agents in cells, tissues, or the whole body. The major secondary metabolites from plants are polyphenols, which have been reported to possess antioxidant and free radical scavenging activities. Several studies have proven that polyphenols reduce the risk of degenerative diseases by diminishing oxidative stress and inhibiting macromolecular oxidation (Silva et al., 2004). The highest concentration of total phenolics was measured in AC (900.91 ± 22.81 μg), followed by AI (419.35 ± 43.88 μg) and AA (392.13 ± 25.61 μg) of GAE/g DW. Flavonoids make up the most common group of plant polyphenols, with a wide range of biological activities, which includes antibacterial, antiviral, anti-inflammatory, anti-allergic, and vasodilatory actions (Abdella et al., 2009). The maximum concentration of total flavonoids was measured in AC (2918.83 ± 109.37 μg), followed by AI (1852.31 ± 240.81 μg) and AA (1422.85 ± 80.61 μg) of QE/g DW. The results are shown in Table 1.

3.2. Antioxidant assays

3.2.1. DPPH assay

DPPH is a stable organic nitrogen free radical that has been widely used to study the antioxidant capacity of plant extracts or compounds. In this assay, picryl hydrazyl radical (purple color) was reduced to picryl hydrazine (pale yellow color) by plant extracts or antioxidant compounds (Blois, 1958). The color change or discoloration indicates the free radical scavenging activity of the tested sample. The capability of DPPH reduction was determined by the decrease in its absorbance at 517 nm, which is roused by antioxidants. In the DPPH assay, all three *Artemisia* species showed significant radical scavenging effects with increasing concentration. The studied plant extracts showed scavenging activity in a concentration-dependent manner. The investigated plant extracts demonstrated the lowest IC_{50} value of 0.14 ± 0.02 mg/mL for AC, followed by 0.17 ± 0.01 mg/mL for AI and 0.35 ± 0.03 mg/mL for AA. Ascorbic acid and gallic acid were used as standard and showed IC_{50} values of 0.001 ± 0.0 mg/mL and 0.006 ± 0.0 mg/mL, respectively (Table 2). On average, the DPPH radical scavenging activities were in the order of *A. capillaris* > *A. iwayomogi* > *A. annua*.

3.2.2. Superoxide scavenging assay

The scavenging of superoxide radicals is important because it is one of the precursors of the singlet oxygen and hydroxyl radicals. (Marklund and Marklund, 1974). Oxidation reactions at the cellular level, superoxide radicals, are normally formed first, and their effects can be magnified because they produce other kinds of cell damaging free radicals and oxidizing agents (Aruoma 1996a,). Different concentrations of three *Artemisia* species were used to measure the scavenging activities of superoxide radicals. In our results, marked inhibitory effects of all *Artemisia* aqueous extracts on superoxide radicals were shown in a dose-dependent manner. The order of IC_{50} values was shown to be 8.66 ± 0.06, 8.54 ± 0.42, and 9.42 ± 0.06 mg/mL for AC, AI, and AA, respectively (Table 2). Ascorbic acid and gallic acid were used as standard and investigated under the same conditions, ultimately showing IC_{50} values of 0.008 ± 0.0 mg/mL and 0.02 ± 0.01 mg/mL, respectively.

3.2.3. Hydroxyl ion scavenging assay

The hydroxyl radical is an extremely reactive free radical formed in biological systems that has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells (Hochestein and Atallah, 1988). This radical has the capacity to join nucleotides in DNA and can cause strand breakage, which contributes to carcinogenesis, mutagenesis, and cytotoxicity (Manian et al., 2008). The Fenton reaction generates hydroxyl radicals which degrade DNA deoxyribose by using Fe^{2+} salts as an important catalytic component. Oxygen radicals may attack DNA at either the sugar or the base, giving rise to a large number of products (Rajeshwar et al., 2005). The lowest IC_{50} value with highest scavenging capacity was found to be 3.85 ± 0.06 mg/mL for AC, followed by 7.81 ± 0.14 mg/mL for AI and 8.66 ± 0.02 mg/mL for AA (Table 2). The standard gallic acid had an IC_{50} value of 0.06 ± 0.0 mg/mL.

3.2.4. Nitric oxide scavenging assay

Nitric oxide is an important bioregulatory molecule involved in several physiological processes like neural signal transmission, immune

Table 1
Total phenolic, flavonoids and antioxidant capacity.

Artemisia species	TPC (μg/g of GAE)	TFC (μg/g of QE)	TAC (μg/g of GAE)	FRAP (μg/g of $FeSO_4$)
Artemisia capillaris	900.91 ± 22.81	2918.83 ± 109.37	43.21 ± 0.28	3880.24 ± 340.37
Artemisia iwayomogi	419.35 ± 43.88	1852.31 ± 240.81	25.42 ± 6.55	3599.33 ± 435.78
Artemisia annua	392.13 ± 25.61	1422.85 ± 80.61	20.08 ± 3.25	2474.56 ± 49.65

*All the SD values given were the results of triplicate estimates each study, n = 3.

Table 2
Antioxidant activities of plant extracts and standards.

Plant or Drug name	IC_{50} (mg/ml)					
	DPPH	H_2O_2	Nitric oxide	Super oxide	Hydroxyl	Iron chelating
Artemisia capillaris	0.14 ± 0.02	0.01 ± 0.00	1.05 ± 1.75	8.66 ± 0.06	3.85 ± 0.01	ND
Artemisia iwayomogi	0.17 ± 0.01	0.02 ± 0.00	1.17 ± 0.42	8.54 ± 0.42	7.81 ± 0.14	3.83 ± 0.06
Artemisia annua	0.35 ± 0.03	0.04 ± 0.00	3.14 ± 0.30	9.42 ± 0.06	8.66 ± 0.02	4.78 ± 0.1
Ascorbic acid	0.001 ± 0.0	ND	ND	0.008 ± 0.0	ND	ND
BHT	ND	0.03 ± 0.00	ND	ND	ND	ND
EDTA	ND	ND	ND	ND	ND	0.06 ± 0.00
Gallic acid	0.006 ± 0.0	0.002 ± 0.0	0.05 ± 0.00	0.02 ± 0.01	0.06 ± 0.00	ND

*All the SD values given were the results of triplicate estimates each study, n = 3.

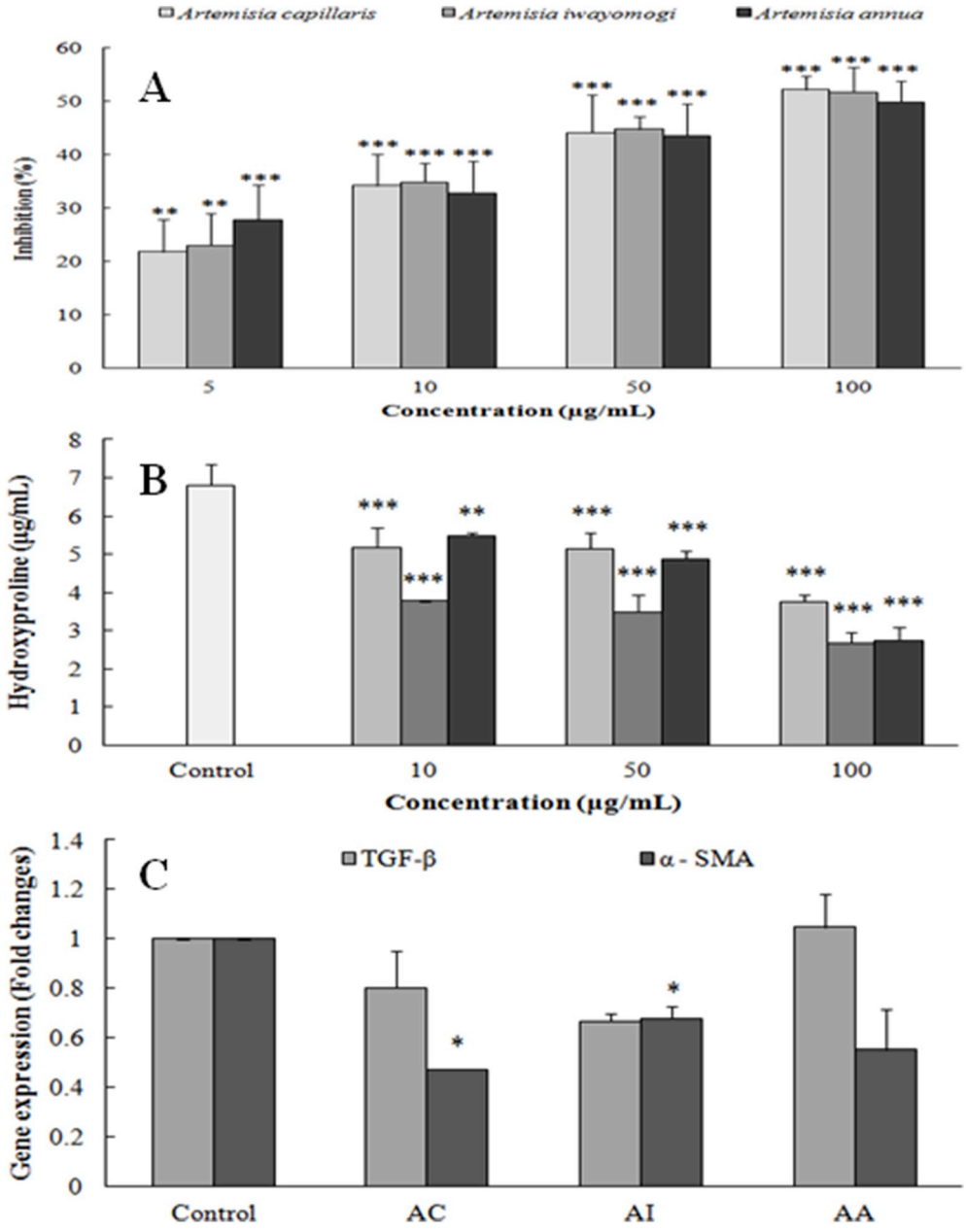

Fig. 1. Effect of Artemisia species on cell proliferation, hydroxyproline content and gene expression. (A) Inhibition percentage of activated HSC's treated with 5, 10, 50, 100 μg/mL. (B) Hydroxyproline content in Artemisia species. (C) Alterations in the gene expression of TGF - β and α - SMA determined by RT-PCR. The gene expressions were normalized to that of β-actin. Data are expressed as the means ± *SDs*. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the control group. AC = *Artemisia capillaris*, AI = *Artemisia iwayomogi*, AA = *Artemisia annua*.

response, control of vasodilatation, and blood pressure (Jagetia et al., 2002). In the present study, our results indicated that the lowest IC_{50} value was shown in AC (1.05 ± 0.75 mg/mL), followed by AI (1.17 ± 0.42 mg/mL) and AA (3.14 ± 0.30 mg/mL) (Table 2). Gallic acid was used as standard, and showed an IC_{50} value of 0.05 ± 0.0.

3.2.5. Hydrogen peroxide scavenging assay

Hydrogen peroxide (H_2O_2) was generated in large quantity during the inflammatory process, which may be due to the activation of mast cells, macrophages, eosinophils, and neutrophils, which generate superoxide radicals, predominantly via NADPH oxidase (Barnes, 1990; Hamelmann et al., 1999). The superoxide is then rapidly converted into H_2O_2 by superoxide dismutase (SOD). H_2O_2 can easily enter the membranes of surrounding cells, whereas superoxide usually cannot. In our study, all three *Artemisia* species were confirmed to show scavenging property, and they exhibited IC_{50} values of 0.01 ± 0.0, 0.02 ± 0.0, and 0.04 ± 0.0 mg/mL for AC, AI, and AA, respectively (Table 2). Gallic acid and BHT were used as standard and showed IC_{50} values of 0.002 ± 0.0 and 0.03 ± 0.0 mg/mL, respectively.

3.2.6. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay is commonly used in routine analysis to evaluate antioxidant capacity, since it is a rapid, simple, sensitive, and reproducible method (Prior et al., 2005). The assay is based on the ability of antioxidant compounds to reduce complex ferric (Fe (III)–TPTZ) to ferrous (Fe (II)–TPTZ). The Fe (II)–TPTZ complex gives a blue color with an absorbance maximum at 593 nm (Zou et al., 2011). In order to determine the antioxidant capacities of the samples, the absorbance values were compared with those obtained from the linear standard curves of $FeSO_4$ ($r^2 = 0.9971$). The antioxidant capacity values were expressed as μg $FeSO_4$ equivalent in g extract (μg $FeSO_4$ eq/g extract). Within these plants, the AC displayed the highest FRAP value (3880.24 ± 340.37), followed by AI (3599.33 ± 435.78) and AA (2474.56 ± 49.65 μg of FeSO4 eq/g) (Table 1).

3.2.7. Total antioxidant capacity (TAC)

There are actually three TAC tests developed at different periods, namely TAC I ($ABTS^{\cdot+}$ generated enzymatically with metmyoglobin and hydrogen peroxide), TAC II (radical generation with filtration over the MnO_2 oxidant), and TAC III (with potassium per sulphate ($K_2S_2O_8$) oxidant), which were totally different from each other and applicable to different solvent media, and their findings for a given antioxidant could vary significantly (Schleiser et al., 2002). The advantages of ABTS/TAC have been reported to be their operational simplicity, reproducibility, diversity, and flexible usage in multiple media, since the reagent is soluble in both aqueous and organic solvent media (Awika et al., 2003). The calibration curve was plotted using gallic acid ($r^2 = 0.998$) and the results were expressed as μg of GA eq/g. Based on these plants, the highest antioxidant capacity was observed in AC (43.21 ± 0.277), followed by AI (25.42 ± 6.55) and AA (20.08 ± 3.25 μg of GA eq/g).

3.2.8. Iron chelating assay

Among the transition metals, iron is known to be the most important lipid oxidation pro-oxidant, due to its high reactivity. The ferrous state of iron accelerates lipid oxidation by breaking down hydrogen and lipid peroxides to reactive free radicals via the Fenton reaction. Fe^{3+} ion also produces radicals from peroxides, although the rate is less than that of Fe^{2+} ion. Thus, Fe^{2+} ion is the most powerful pro-oxidant among the various species of metal ions (Lim et al., 2002). The highest chelation with a lowest IC_{50} was observed in AI (3.83 ± 0.06), followed by AA (4.78 ± 0.1 mg/mL) (Table 2). AC did not show 50% inhibition in all tested concentrations. The EDTA was used as standard, and showed an IC_{50} value of 0.06 ± 0.0 mg/mL.

3.3. Anti fibrotic activity

3.3.1. Anti proliferation assay

We hypothesized three *Artemisia* species for anti proliferation by assessing the cell viability in fully-activated rat HSC's. Among the plants tested, AC and AA displayed the most potent inhibitory activities on the proliferation of HSCs after 48 h of incubation. Our present results revealed that all species showed significantly decreased HSC proliferation (AC and AI = $p < 0.01$ and 0.001, AA = $p < 0.001$) in a concentration-dependent manner (Fig. 1A). The AI showed weak inhibitory activity as compared with other plants. Since the plants tested the inhibitory activity on HSC proliferation, we further observed the cell morphology under a phase contrast microscope. HSCs cultured in the absence of plant extracts exhibited flattened and membranous processes, representing myofibroblastic morphology. However, the morphology of HSCs treated with plant extracts changed to a slender cell shape at the tested concentrations (Fig. 2).

3.3.2. Hydroxyproline content

In order to assess the effect of Artemisia extracts on ECM

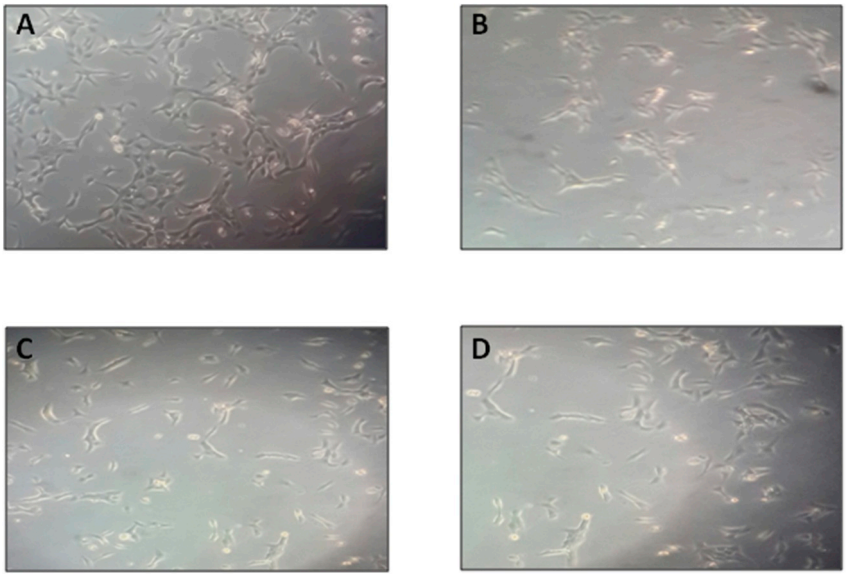

Fig. 2. Effect of Artemisia species on cell morphology in HSC-T6 cells. HSC-T6 cells were incubated with vehicle (control) (A), *Artemisia capillaris* (B), *Artemisia iwayomogi* (C) and *Artemisia annua* (D) for 48 h at 100μg/mL. Cells were observed with phase contrast microscope (original magnification X 45).

production, the hydroxyproline content was measured. Treatment with different concentrations of extracts significantly reduced the cell hydroxyproline content in a dose-dependent manner as compared to control. The inhibition of hydroxyproline content was significantly reduced for AI (2.68 ± 0.28 μg/mL) ($p < 0.001$), followed by AA (2.74 ± 0.36 μg/mL) ($p < 0.01$, 0.001) and AC (3.76 ± 0.17 μg/mL) ($p < 0.001$) at 100 μg/mL (Fig. 1B).

3.3.3. Gene expression analysis by qRT-PCR

Following the demonstration of the growth inhibitory effect of three *Artemisia* species on activated HSCs, we continued to further examine these plant extracts (50 μg/mL) in terms of mRNA expression of two main fibrosis related genes, including TGF - β and α-SMA in cultured activated rat HSCs. The addition of extracts significantly downregulated the TGF - β and α-SMA ($p < 0.05$) as compared with control (Fig. 1C).

3.3.4. Correlation between total phenolic/flavonoid contents and antioxidant activity

The antioxidant activities of plants containing polyphenolic compounds are mainly due to their ability to act as hydrogen donors, reducing agents, and radical scavengers (Niciforovic et al., 2010). The correlation between phenolic contents and antioxidant properties was determined by using Pearson correlation coefficient (r), and the results are shown in Table 3. According to these results, the higher correlation was found for all species between total phenolics and total flavonoids with FRAP than those of other methods. Sulaiman et al. (2011) reported the highest correlations between TPC and FRAP with the extracts of *Coriandrum sativum*. In the three species, the highest correlation was found between FRAP and total flavonoids ($r = 0.998$, $p < 0.01$) with the extracts of AA. Meanwhile, the values of total phenolics of AI and AA were significantly correlated with superoxide scavenging assay ($r = 0.991$, $p < 0.01$). The lowest correlation was found between total flavonoids and TAC ($r = 0.815$, $p < 0.05$). There were no statistically significant correlations between total phenolics/flavonoids content and H_2O_2. These findings suggest that the activities of extracts on these methods may be attributable to the presence of non-phenolic compounds.

4. Discussion

Recently, several methods have been employed for the determination of antioxidant activities. The chemical compositions of extracts, often a mixture of dozens of compounds with different functional groups, polarities, and chemical behaviors, could lead to uneven results, depending on the test employed. Therefore, a reliable approach for evaluating the antioxidant potential of extracts with multiple assays would be more informative and even necessary. In our study, the eight methods of DPPH, superoxide anion, hydroxyl, nitric oxide radical scavenging activity, metal chelating activity, ferric reducing antioxidant power, and total antioxidant capacity were mainly employed. The concentrations of total phenolic and flavonoids were also determined for the tested extracts.

In the present study, three different aqueous extracts of artemisia often used in Korean Traditional Medicine (KTM) have been tested for their antioxidant activity by measuring their ability to inhibit the free radicals. The three extracts showed different capacities to inhibit free radicals. At a given concentration, the inhibiting capacity for different extracts is in the order AC > AI > AA. The effective inhibiting concentrations corresponding to 50% (IC_{50} values) of AC were obviously lower than those of AI and AA. These results confirmed that all of the plant extracts tested exhibit some antioxidant potential, but to different extents. There are a number of mechanisms by which antioxidants can act. One of them is by the scavenging of reactive oxygen (ROS) and nitrogen free radicals (RNS). There are many different testing methods by which the free radical scavenging activity can be estimated. One such method involves determining their efficiency to scavenge DPPH radicals. DPPH radical is a stable free radical, and any molecule that can donate an electron or hydrogen to DPPH can react with it, and thereby lighten or remove the DPPH color (Blois, 1958). When the above extracts were tested for DPPH scavenging ability, only AC and AI showed high activity with the lowest IC_{50} values as compared to those of AA. These results suggested that all Artemisia species contain compounds such as polyphenols that can easily donate electrons/hydrogen, but to different extents.

In order to further confirm the radical scavenging activity, we used H_2O_2, Superoxide, Hydroxyl, Nitric oxide, and iron chelating ability. The results of these methods were in close agreement with the DPPH scavenging study, excluding metal chelating activity. Furthermore, we confirmed the antioxidant abilities in terms of μg/g of different standard equivalents. In TAC, the gallic acid was used as standard and the values obtained were expressed in μg/g of GAE. Among these extracts, AC showed a very high level of total antioxidant capacity in terms of gallic acid equivalents; AI showed a moderate value and AA showed very little value. The ferric reducing antioxidant power (FRAP) of tested extracts were steadily increased with increasing sample concentration. All extracts, at testing concentrations, were capable of reducing Fe^{3+}. Similar to the other results obtained, the strongest reducing power was found for AC at a concentration of 10 mg/mL followed by AI, while the weakest reducing power was found in AA at the same concentration. Hong et al. showed the antioxidant activity of different solvent fractions of *Artemisia capillaris* and its ethyl acetate fraction showed lower IC_{50} values in DPPH, Nitric oxide, TBARS, and superoxide scavenging activity. The chloroform fraction of *Artemisia capillaris* showed a smaller IC_{50} value in hydroxyl scavenging activity (Hong et al., 2009). Kim et al. showed strong DPPH scavenging activity in ethyl acetate fraction of *Artemisia iwayomogi*. Furthermore, different compounds were isolated, but chlorogenic acid isolated from n-butanol fraction of methanolic extract of *Artemisia iwayomogi* exhibited higher scavenging ability on DPPH(Kim et al., 2003). Isabel et al. compared the antioxidant profiles of six artemisia species, and *Artemisia annua* showed substantially less antioxidant capacity than the other species (Isabel et al., 2011). The same findings were observed in our results, that *Artemisia annua* displayed less antioxidant potential than other species. A comparison of our results with those of previous studies shows that the

Table 3
Pearson's correlation coefficients of total phenolics, total flavonoids and antioxidant.

Methods	AC		AI		AA	
	Total phenols	Total flavonoids	Total phenols	Total flavonoids	Total phenols	Total flavonoids
DPPH	0.975**	0.867*	0.977**	0.884*	0.985**	0.958**
Superoxide	0.987**	0.909*	0.991**	0.918**	0.991**	0.963**
Hydroxyl	0.930**	0.797	0.909*	0.796	0.939**	0.909*
Nitric oxide	0.951**	0.911**	0.987**	0.948**	0.951**	0.973**
H_2O_2	0.622	0.456	0.712	0.560	0.773	0.721
FRAP	0.993**	0.996**	0.916**	0.997**	0.948**	0.998**
TAC	0.834*	0.673	0.941**	0.815*	0.926**	0.872*
Iron chelating	0.977**	0.985**	0.853*	0.980**	0.898**	0.903**

*Significant at $p < 0.05$,** Significant $p < 0.01$.

locality of the plant material and the extraction procedure leads to differences in the antioxidant activities of the plants.

The activation of HSCs plays a major role in myofibroblast proliferation for the development of liver fibrosis. In our study, we evaluated the antiproliferation and anti-fibrotic potential of three Artemisia species in activated HSC cells. The results showed that the growth of HSCs was inhibited by Artemisia extracts, and comprises the first piece of evidence for an antiproliferative effect of these extracts. We proved that Artemisia extracts decreases the proliferation of HSC's, as shown by the spectrophotometric determination of HSC's growth. Cell growth inhibition was significantly improved by the addition of increasing concentrations of Artemisia extracts. These data suggest that Artemisia extracts may play a key role as a negative regulator of liver fibrogenesis. Recently, several reports have demonstrated that natural dietary plants may play an antioxidative role in the prevention of aging and carcinogenesis, and may also offer effective protection from lipid peroxidative damage *in vitro* and *in vivo* (Galli and Costa, 1995; Hamelmann et al., 1999). The Artemisia extracts directly inhibited the proliferation of HSCs and induced cell death in a dose-dependent manner. As shown in Fig. 2, the treatments of AC, AI, and AA (100 μg/mL) changed the cell morphology from flattened myofibroblastic membranous morphology, indicating an activation state, to a slender shape, indicating a quiescent state. As compared with the antioxidant results, the HSC – T6 proliferation was in same order of AC > AI > AA.

Furthermore, we used an activated HSC-T6 cell line for the assessment of antifibrotic activity in terms of measuring hydroxyproline content. Hydroxyproline, a main component of the protein collagen, is produced by the hydroxylation of the amino acid proline by the enzyme prolyl hydroxylase following protein synthesis. In order to evaluate extracellular matrix production, the hydroxyproline content was measured. The cell hydroxyproline content was higher in activated HSC-T6 cell line, and this was reversed or inversed in a dose-dependent manner after the addition of Artemisia extracts.

In liver fibrosis, the cytokines play a major role in activating the HSCs. These activated HSCs becoming a main source for the stimulation of cytokines leads to collagen production. Among the cytokines, TGF-β1 is the most potent fibrogenic cytokine in liver fibrosis, and its expression is up-regulated during fibrogenesis, which induces increased ECM synthesis (Shek and Benyon, 2004). The primary approach to treating liver fibrosis aims to disrupt or decrease TGF-β1 expression. Alpha smooth muscle actin (α-SMA) is a commonly used marker of myofibroblast formation, and the TGF-β/α-SMA pathway is the most potent fibrogenic stimulus to HSCs, resulting in increased production of ECM (Lin et al., 2013). We investigated the Artemisia species for the inhibition of the development of hepatic fibrosis in terms of TGF-β 1, as well as α-SMA gene expression levels in cells treated or not treated with extracts. The results showed that the treatment of all extracts markedly ameliorated the TGF-β1 and α-SMA mRNA gene expression levels in activated rat HSCs. This was treated as further evidence that TGF β secretion during cell growth triggers liver fibrosis by means of collagen accumulation.

Our results collectively demonstrated that, all Artemisia extracts possess prominent anti-proliferation as well as anti-fibrotic activities via the inhibition of TGF- β gene expression in activated HSCs. On the other hand, the positive correlations between antioxidant assays and anti fibrotic activity suggest its involvement in liver regeneration.

5. Conclusion

In conclusion, our results demonstrated that all three *Artemisia* extracts exhibited significant antioxidant potentials. Of the three plants, *Artemisia capillaris* and *Artemisia iwayomogi* were found to offer the most antioxidant capacity with lower IC_{50} values than *Artemisia annua*. In addition, antiproliferative, anti-fibrotic, and mRNA gene expression data showed that AC and AI remarkably inhibited or inversed liver fibrogenesis by downregulating TGF-β1 and α-SMA gene expression, which are commonly used markers for myofibroblast formation. The possible mechanism associated with this anti-fibrotic potential may involve the inhibition of TGF-β/Smad pathway. Thus, we suggest that AC and AI may be useful in preventing the development of liver fibrosis. This will provide further insight into the design of new approaches to liver fibrosis.

Acknowledgement

We authors of the manuscript express our sincere thanks to Prof Dr. Yong Soo Kwon, College of Pharmacy, Kangwon National University Korea. for his help in identifying the plant and their species.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101040.

References

Abdella, E.M., Tohamy, A., Ahmad, R.R., 2009. Antimutagenic activity of Egyptian propolis and bee pollen water extracts against cisplatin induced chromosomal abnormalities in bone marrow cells of mice. Iran. J. Cancer Prev. 2, 175–181.
Aruoma, O., 1996a. Assessment of potential prooxidant and antioxidant actions. J. Am. Oil Chem. Soc. 73, 1617–1625.
Awika, J.M., Rooney, L.W., Wu, X., Prior, R.L., Cisneros Zevallos, L., 2003. Screening methods to measure antioxidant activity of Sorghum (Sorghum bicolor) and Sorghum products. J. Agric. Food Chem. 51, 6657–6662.
Barnes, P.J., 1990. Reactive oxygen species and airway inflammation. Free Radic. Biol. Med. 9, 235–243.
Bataller, R., Brenner, D.A., 2005. Liver fibrosis. J. Clin. Invest. 115, 209–218.
Benzie, I.F.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. Anal. Biochem. 239, 70–76.
Bhattarai, A., Ali, A.S., Kachur, S.P., et al., 2007. Impact of artemisinin based combination therapy and insecticide treated nets on malaria burden in Zanzibar. PLoS Med. 4, 1784–1790.
Blois, M.S., 1958. Antioxidant determinations by the use of a stable free radical. Nature 181, 1199–1201.
Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of free radical method to evaluate antioxidant activity. LWT (Lebensm.-Wiss. & Technol.) 28, 25–30.
Choi, W.S., Kim, C.J., Park, B.S., Lee, S.E., Takeoka, G.R., Kim, D.G., Lanpiao, X., Kim, J.H., 2005. Inhibitory effect on proliferation of vascular smooth muscle cells and protective effect on CCl_4 induced hepatic damage of HEAI extract. J. Ethnopharmacol. 100, 176–179.
Dinis, T.C.P., Madeira, V.M.C., Almeida, L.M., 1994. Action of phenolic derivates (acetoaminophen, salycilate, and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys. 315, 161–169.
Elizabeth, K., Rao, M.N.A., 1990. Oxygen radical scavenging activity of curcumin. Int. J. Pharmaceutics. 58, 237–240.
Friedman, S.L., 2008a. Hepatic stellate cells: protean, multifunctional and enigmatic cells of the liver. Physiol. Rev. 88, 125–172.
Friedman, S.L., 2008b. Mechanisms of hepatic fibrogenesis. Gastroenterol. 134, 1655–1669.
Galli, S., Costa, J.J., 1995. Mast-cell-leukocyte cytokine cascades in allergic inflammation. Allergy 50, 851–857.
Hamelmann, E., Tadeda, K., Oshiba, A., Gelfand, E.W., 1999. Role of IgE in the development of allergic airway inflammation and airway hyper responsiveness a murine model. Allergy 54, 297–302.
Henderson, N.C., Iredale, J.P., 2007. Liver fibrosis: cellular mechanisms of progression and resolution. Clin. Sci. 112, 265–280.
Hochestein, P., Atallah, A.S., 1988. The nature of oxidant and antioxidant systems in the inhibition of mutation and cancer. Mutat. Res. 202, 363–375.
Hong, J.H., Hwang, E.Y., Kim, H.J., Jeong, Y.J., Lee, I.S., 2009. Artemisia capillaris inhibits lipid accumulation in 3T3-L1adipocytes and obesity in C57BL/6J mice fed a high fat diet. J. Med. Food 12, 736–745.
Hong, J.H., Lee, I.S., 2009. Effects of Artemisia capillaris ethyl acetate fraction on oxidative stress and antioxidant enzyme in high fat diet induced obese mice. Chem. Biol. Interact. 179, 88–93.
Hsu, E., 2006. The history of qing hao in the Chinese materia medica. Trans. R. Soc. Trop. Med. Hyg. 100, 505–508.
Isabel, S. Carvalho, Teresa, Cavalho., Maria, Brodelius., 2011. Phenolic composition and antioxidant capacity of six artemisia species. Ind. Crops and Prod 33, 382–388.
Jagetia, G.C., Baliga, M.S., Malagi, K.J., Kamath, M.S., 2002. The evaluation of the radioprotective effect of Triphala (an ayurvedic rejuvenating drug) in the mice exposed to γ- radiation. Phytomedicine 9, 99–108.
Jang, G., 1975. Jung Gyeong Jeon Seo. Publishers, Seoul, Hollym Corp.
Kim, D.O., Jeong, S.W., Lee, C.Y., 2003. Antioxidant capacity of phenolic phytochemicals from various cultivars of plum. Food Chem. 81, 321–326.
Lee, J., Koo, N., Min, D.B., 2004. Reactive oxygen species, aging, and antioxidative

nutraceuticals. Compr. Rev. Food Sci. Food Saf. 3, 21–33.
Lee, T.Y., Chang, H.H., Chen, J.H., Hsueh, M.L., Kuo, J.J., 2007. Herb medicine Yin Chen Hao Tang ameliorates hepatic fibrosis in bile duct ligation rats. J. Ethnopharmacol. 109, 318–324.
Lee, W.T., 1996. Standard Illustrations of Korean Plants. Academy publishing, Seoul Korea, pp. 345–349.
Lim, S.N., Cheung, P.C.K., Ooi, V.E.C., Ang, P.O., 2002. Evaluation of antioxidative activity of extracts from a brown seaweed Sargassum siliquastrum. J. Agric. Food Chem. 50, 3862–3866.
Lin, Liu, Xue-mei, Li, Liang, Chen, Qin, Feng, Lili, Xu, Yi-yang, Hu, Jing-hua, Peng, 2013. The effect of gypenosides on TGF-β1/smad pathway in liver fibrosis induced by carbon tetrachloride in rats. Int. J. Integr. Med. 1, 1–6.
Manian, R., Anusuya, N., Siddhuraju, P., Manian, S., 2008. The antioxidant activity and free radical scavenging potential of two different solvent extracts of *Camellia sinensis (L.)* O. *Kuntz, Ficus bengalensis L.* and *Ficus racemosa L.* Food Chem. 107, 1000–1007.
Mantena, R.K.R., Wijburgm, O.L.C., Vindurampulle, C., et al., 2008. Reactive oxygen species are the major antibacterials against Salmonella typhimurium purine auxotrophs in the phagosome of RAW 264.7 cells. Cell Microbiol. 10, 1058–1073.
Marklund, S., Marklund, G., 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem. 47, 469–474.
Muller, H.E., 1985. Detection of hydrogen peroxide produced by microorganism on ABTS-peroxidase medium. Zentrabl. Bakteriol. Mikrobiol. Hyg. A. 259, 151–154.
Nam, S.M., Ham, S.S., Oh, D.H., Kang, I.J., Lee, S.Y., et al., 1998. Effects of Artemisia iwayomogi KITAMURA ethanol extracts on lowering serum and liver lipids in rats. J. Kor. Soc. Food. Sci. Nutri. 27, 201–365.
Niciforovic, N., Mihailovic, V., Maskovic, P., et al., 2010. Antioxidant activity of selected plant species; potential new sources of natural antioxidants. Food Chem. Toxicol. 48, 3125–3130.
Noori, S., Dawood, Al-Waili, 1986. Treatment of diabetes mellitus by Artemisia herba-alba extract: preliminary study. Clin. Exp. Pharmacol. Physiol. 13, 569–574.
Prior, R.L., Wu, X., Schaich, K., 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J. Agric. Food Chem. 53, 4290–4302.
Rajeshwar, Y., Kumar, G.P., Gupta, M., Mazumder, U.K., 2005. Studies on *In vitro* antioxidant activities of methanol extract of *Mucuna pruriens* (Fabaceae) seeds. Eur. Bull. Drug. Res. 13, 31–39.
Ryu, J.H., Ahn, H., Kim, J.Y., Kim, Y.K., 2003. Inhibitory activity of plant extracts on nitric oxide synthesis in LPS- activated macrophages. Phytother Res. 17, 485–489.
Schleiser, K., Harwat, M., Bohm, V., Bitsh, R., 2002. Assessment of antioxidant activity by using different in vitro methods. Free Radic. Res. 36, 177–187.
Seo, K.S., Jeong, H.J., Yun, K.W., 2010. Antimicrobial activity and chemical components of two plants, Artemisia capillaris and Artemisia iwayomogi used as Korean herbal Injin. J. Ecol. Field. Biol. 33, 141–147.
Seo, K.S., Yun, K.W., 2008. Antioxidant activities of extracts from artemisia capillaris thunb. And artemisia iwayomogi Kitam. Used as injin. Korean J. Polar Res. 21, 292–298.
Shek, F.W., Benyon, R.C., 2004. How can transforming growth factor beta be targeted usefully to combat liver fibrosis. Eur. J. Gastroenterol. Hepatol. 16, 123–126.
Shin, T.Y., Park, J.S., Kim, S.H., 2006. Artemisia iwayomogi inhibits immediate type allergic reaction and inflammatory cytokine secretion. Immunopharmacol. Immunotoxicol. 28, 421–430.
Sikorski, Z.E., 2001. Chemical and Function Properties of Food Components. CRC Press, New York.
Silva, B.M., Andrade, P.B., Valentao, P., 2004. Quince (Cydonia oblonga Miller) fruit (pulp peel and seed) and jam: antioxidant activity. J. Agric. Food Chem. 52, 4705–4712.
Smirnoff, N., Cumbes, Q.J., 1989. Hydroxyl radical scavenging activity of compatible solutes. Phytochem 28, 1057–1060.
Sreejayan, Rao, M.N.A., 1997. Nitric oxide scavenging by curcuminoids. J. Pharm. Pharmacol. 49, 105–107.
Sulaiman, S.F., Abu, B.S.A., Kheng, L.O., Meng, S.E., 2011. Effect of solvents in extracting polyphenols and antioxidants of selected raw vegetables. J. Food Compos. Anal 24, 506–515.
Valentao, P., Fernandes, E., Carvalho, F., Andrade, P.B., Seabra, R.M., 2002. Antioxidant properties of cardoon (*Cynara cardunculus* L.) infusion against superoxide radical, hydroxyl radical and hypochlorous acid. J. Agric. Food Chem. 50, 4989–4993.
Wang, J.H., Choi, M.K., Shin, J.W., Hwang, S.Y., Son, C.G., 2012. Antifibrotic effects of *Artemisia capillaris* and *Artemisia iwayomogi* in a carbon tetrachloride induced chronic hepatic fibrosis animal model. J. Ethnopharmacol. 140, 179–185.
William, T.C., Jianping, Y., Aamir, Z., David, M.R., Ilya, R., 2008. Botanicals and the metabolic syndrome. Am. J. Clin. Nutr. 87, 481s–487s.
Wright, C.W., Linley, P.A., Brun, R., Wittlin, S., Hsu, E., 2010. Ancient Chinese methods are remarkably effective for the preparation of artemisinin rich extracts of Qing Hao with potent antimalarial activity. Molecules 15, 804–812.
Wu, J., Zern, M.A., 2000. Hepatic stellate cells: a target for the treatment of liver fibrosis. J. Gastroenterol. 35, 665–672.
Zou, Y., Chang, S.K.C., Gu, Y., Qian, S.Y., 2011. Antioxidant activity and phenolic compositions of lentil (*Lens culinaris* var. Morton) extract and its fractions. J. Agricul. Food. Sci. 58, 2268–2276.