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Inhibition of hepatitis C virus (HCV) genotype 2a RNA replication by extracts of medicinal herbs with anti-cholesterol effects

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Hepatitis C virus (HCV) is a worldwide disease agent causing chronic hepatitis and hepatocellular carcinoma. Nevertheless, there are few HCV drugs such as interferon and ribavirin with serious side effects. The purpose of this study is to find a multi-functional herb to inhibit HCV replication among medicinal herbs. Twenty nine medicinal herb extracts were selected. The functional medicinal herbs were tested for inhibition of HCV replication in HCV genotype 2a producing cell line. The stem and flower of *Eupatorium chinensis* var. *simplicifolium* showed high anti-bacterial effects to *Staphylococcus aureus* and *Candida albicans,* but not to *Escherichia coli.* The stem and flower of *Oenothera odorata* showed high anti-oxidant effects which is similar to those of quercetin and phloroglucinol used as positive controls. The stem of *Anethum graveolens* and the flower of *Celosia cristata,* Red was high cholesterol adhesion effect among all extracts. Among the six selected herbs, the flower of *Celosia cristata,* Red inhibited HCV 2a RNA replication, significantly. This result indicates that the flower of *Celosia cristata,* Red is one of candidate drugs to treat HCV infection and high cholesterolemia. However, a useful single component from the flower of *Celosia cristata,* Red should be isolated and more studied for clinical use.

Key words: Hepatitis C virus, medicinal herb, anti HCV drug, anti-cholesterol effect.

INTRODUCTION

Hepatitis C virus (HCV) is a worldwide disease agent causing liver disease. Approximately 3% of world populations (almost 1.8 millions) were infected with HCV in the world (WHO, 2011). In Korea, approximately 6000 cases were reported annually (KCDC, 2008). It causes chronic hepatitis and develops liver cirrhosis and hepato-cellular carcinoma. Fifty five to eighty five percent of acute HCV infected persons become chronic infection. Seventy percent of chronically infected persons undergo liver disease and five percent of them develop cancer. Its incubation period is ten to twenty years. Considering discovery time, we expect that HCV attack rate will increase (CDC, 2005; Seeff and Hoofnagle, 2002).

HCV belongs to the Flaviviridae having positive-sense RNA genome. It is 9.6 kb nucleotides long and encodes a precursor polyprotein cleaved by cellular peptidase and viral protease into structural protein (Core, E1, E2 and p7) and nonstructural protein (NS2, NS3, NS4A, NS4B, NS5A and NS5B) (Grakoui et al., 1993; Rehman et al., 2011b). The genotypes are classified by a little variation of this gene. In Korea, genotype 1b and 2a are detected mostly (Lim, 2009). A lot of studies investigated that Core,

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NS2, NS3 and NS5 play an important role in replication of HCV (André et al., 2002; Chang et al., 2007; Lorenz et al., 2006).

Oxidative stress induces modern diseases like cancer by nucleic acid transformation, arteriosclerosis by physiological depression, diabetes, cerebral apoplexy, nephritis, atopic dermatitis and aging (Halliwell and Aruoma, 1991; Kanno et al., 2003; Kuroki et al., 2003). In HCV infection, some HCV viral proteins produce oxidative stress and it influence to development hepatitis to hepato-carcinoma. HCV inhibition tests using anti-oxidant are progressing (Koike and Miyoshi, 2006; Nakamura et al., 2010).

HCV virions packaged as lipoviroparticles (LVPs) circulate in blood. Its density is similar to very-low-density-lipoprotein (VLDL). LVPs are rich in triglycerides, apolipoprotein B (apoB) and apolipoprotein E (apoE). Especially, there are many studies about correlation apoE and HCV (Chang, 2007; Hishiki et al., 2010). Thus, studies related lipid and HCV are being progressed using statins which are 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA reductase) inhibitors (Chang and Jo, 2011; Ikeda et al., 2006).

HCV suppresses innate immunity like dendritic cell (DC) and Toll like receptors (TLRs). It can reside in liver for a long time and be easy to be infected by other viruses, fungus and bacteria. Therefore, use of antibiotic is increasing. We expect to find drugs having both HCV inhibition and anti-bacterial and anti-fungal effects (Abe et al., 2007).

Currently, pegylated interferon-α (PEG IFN- α) in combination with ribavirin (RBV) are usually used as standard treatment. Nevertheless it has side effects such as hemolytic anemia and low response rate to genotype 2 and 3. In addition, ribavirin is effective to liver state, but it does not work at HCV RNA. Studies about more specific and safe treatments are developing using NS3-4A serine protease, RNA helicase activity of NS3 and NS5B RNA-dependent RNA polymerase (RdRp) inhibitor (Jamall et al., 2008; Rehman et al., 2011a,b). Vaccine development of HCV is difficult, because HCV incubation and cultivation have many problems. HCV animal model is restricted that only chimpanzee is available. In addition, HCV is easily transformed.

Recently, natural extracts are experimented for HCV treatment. Particularly, medicinal herbs are tested actively in Asia. Nevertheless, there is no evidence that which HCV region is target exactly or which compound is effective (Javed et al., 2011; Rehman et al., 2011b). In this study, natural extracts which have effects to anti-bacteria, anti-fungi, anti-oxidant and anti-cholesterol effect in the 29 natural extracts were selected. Fulvic acid, humic acid, phloroglucinol and quercetin were used as single components and controls. Fulvic acid and humic acid belongs to Humic natural complex (HNC). HNC is one hundred percent of natural organism and it was isolated from soil containing deposited plant for 10 million years. It is helpful to plant growth. Especially fulvic acid has anti-bacteria, anti-cancer and anti-inflammatory effect (Yamada et al., 2007). It has anti viral effect against HIV, influenza and SARS virus (Kotwal, 2008). Phloroglucinol and quercetin are known as anti-oxidant. Phloroglucinol has wide range of biological activities in anti-cancer, anti-depressant, anti-microbial, anti-protozoal, anti-spasmodic, anti-viral and anti-inflammation. It is used in cosmetics, textiles, paints, dyeing industries as well as biological activities (Crockett et al., 2008; Singh et al., 2009). Quercetin is isolated frequently from vegetables and fruits and has multi-function. In particular, studies about inhibition of influenza and inflammation are publishing actively (Birt et al., 2009; Kim et al., 2010). In order to find a medical herb to inhibit HCV replication among medicinal herbs having anti-oxidant, anti-cholesterol, anti-bacterial and anti-fungal effects, we treated the selected medicinal herb extracts and natural materials to HCV genotype 2a producing cell line and compared to HCV2a RNA replication in the treated cells.

MATERIALS AND METHODS

Extraction from medicinal herbs

The ten medicinal herbs cultivated by method of Rural development administration (RDA) were harvested at 2009 in Eumseong (GPS : E 128° 62´ N 36° 56´). The herbs were divided into four parts (root, stem, flower and seed) and extracted with methanol (MtOH) three times at room temperature (each time for 3 days). The combined MtOH extracts were then concentrated in vacuum at 40°C, respectively (Table 1). For experiments, fulvic acid, humic acid, phloroglucinol and quercetin were dissolved to 10 mg/ml by MtOH and used as positive control.

Anti-microbial test

Staphylococcus aureus as Gram-positive, *Escherichia coli* as Gram-negative and *Candida albicans* as yeast were used. One colony cultured on blood agar plate (BAP) was incubated in 5 ml brain heart infusion broth (BHI broth) at 37°C for 24 h. Bacterial concentration was measured by observance density (OD) at 600 nm, and diluted up to 0.5×10^4 cell/ml for anti-microbial test. The natural medicinal herb extracts were serially diluted to 10, 100 and 1000 μg/ml, respectively. The diluted extracts and bacteria were replaced in 96-well microplate at same volume. The mixtures were incubated at 37°C for 24 h. BHI broth was used as negative control. The OD was measured at 600 nm using ELISA reader. To confirm bacteria reduction certainly by the extracts, effects of bacterial reduction were measured by colony forming unit (CFU) after treatment of 10 fold diluted samples to 10^6. The treated bacteria, 100 μl, were cultured on BHI agar, and incubated at 37°C for 24 h. In case of *C. albicans*, it was cultured on Sabouraud dextrose agar (SDA) and Sabouraud dextrose broth (SDB), and incubated for 25ºC 48 h.

1,1-Diphenyl-2-picryl hydrazyl (DPPH) radical scavenging ability assay

The extracts from natural extracts were diluted to 100, 1000 μg/ml concentrations in each solvent. 1,1-diphenyl-2-picryl hydrazyl

Table 1. Medicinal herbs used in experiments and their parts and abbreviations.

Medicinal herb	Abbreviation	Part used	Sample name*
Celosia cristata, Red	Cec	Root	Cec-1
		Stem	Cec-2
		Flower	Cec-3
		Seed	Cec-4
Celosia cristata, Yellow	Y-Cec	Root	Y-Cec-1
		Stem	Y-Cec-2
		Flower	Y-Cec-3
Eupatorium chinensis var. *simplicifolium*	Euc	Root	Euc-1
		Stem	Euc-2
		Flower	Euc-3
Eupatorium chinensis. spp	R-Euc	Root	R-Euc-1
		Stem	R-Euc-2
		Flower	R-Euc-3
Oenothera odorata	Evp	Root	Evp-1
		Stem	Evp-2
		Flower	Evp-3
Anethum graveolens	Ang	Root	Ang-1
		Stem	Ang-2
		Flower	Ang-3
Cichorium intybus	Cii	Root	Cii-1
		Stem	Cii-2
Abutilon theophrast Medicus	Aba	Root	Aba-1
		Stem	Aba-2
		Bark of seed	Aba-4
Gomphrena globosa	Gog	Root	Gog-1
		Stem	Gog-2
		Flower	Gog-3
Chenopodium spp	Brc	Root	Brc-1
		Stem	Brc-2

*1, root of herb; 2, stem of herb; 3, flower of herb; 4, seed of herb

(DPPH, 200 μM, Aldrich, USA) was dissolved in ethanol and it was added 190 to 10 μl of sample. After reacted at 37° for 30 min, OD was measured at 550 nm using ELISA reader (Amersham Biosciences, Sweden).

Cell protection assay against oxidative stress

Huh7 (Human hepatoma cell line) cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Thermo Scientific, USA) containing a 10% fetal bovine serum (FBS), 1% nonessential amino acids, 1% penicillin and streptomycin for proliferation at 37℃ and 5% CO_2. The cell (1×10^5 cell/ml) was cultured in 96-well plate at 37℃ and 5% CO_2 for one night. After natural extracts were serially diluted, the 100 μl extracts was added to wells. The mixture was shaken at 150 rpm for 5 min and incubated at 37℃ and 5% CO_2 for 1 day. H_2O_2 (10 mM) was added for inducing oxidative stress in the treated cells. The cells were continually incubated at 37℃ and 5% CO_2 for 2 days. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution (5 mg/ml, Amresco, USA) was dissolved in phosphate buffered saline (PBS) and it were added 20 μl. After shaken at 150 rpm for 5 min, it was incubated at 37℃ and 5% CO_2 for 2 h. Medium were deleted completely from the well. The cells were treated by 200 μl dimethyl sulfoxide (DMSO), and shaken at 150 rpm for 5 min. OD was measured at 560 nm using ELISA reader.

Cholesterol adhesion assay

Adhesion abilities to cholesterol of the extracts were measured using modified Lee's method (Lee et al., 2010). It was measured by total cholesterol kit (Asan-Pharm, Seoul, Korea) using enzyme reaction. Briefly, 100 µl of natural extracts (10 mg/ml) and 1.8 mg/ml of cholesterol (Sigma) in methanol were reacted at 20°C for 20 min. In the mixtures, 50 µl of 0.1 M hexadecyl-trimethyl-ammonium bromide (Sigma, USA) were added and mixtures were centrifuged at 4°C for 10 min in 15,000 rpm. After supernatant was collected, it was reacted with enzyme solution at 37°C for 5 min. OD was measured at 500 nm using ELISA reader.

Inhibition of HCV genotype 2a RNA replication

HCV genotype 2a producing cell (HCV2a cell) was constructed by Chang (Chang and Jo, 2011), and maintained in DMEM containing 10% FBS and 5 µg/ml of blasticidin (Invitrogen, USA). Huh 7 cell was used as negative control. HCV2a cell (1 × 10^5 cell/ml) was cultured in six-well plate. After incubating for overnight, the cell was treated by the natural extracts. The concentration of the extracts which cause cytotoxicity was eliminated. After incubating for 3 days, the treated cells were washed by PBS three times. The RNA was isolated from the cells by accuzol™ RNA extraction solution (Bioneer, Korea). The isolated RNA was amplified by reverse transcriptase PCR (RT-PCR) (Bioneer, Korea). Primers were designed: HCV genotype 2a NS3A forward (GTGGACTGGCACCTACATCT), NS3A reverse (AGAGTCTTGTTGCCAGCTCC), β-actin forward (ACCTAACTTGCGCAGAAAAC), β-actin reverse (GGGCACGAAGGCTCATCATT).

RESULTS

Anti-microbial effects of extracts of medicinal herbs

Twenty nine medicinal herb extracts and four natural materials were used to anti-bacterial effects to *S. aureus.* They have almost anti-bacterial effect weakly. Y-Cec and R-Euc did not have anti-bacteria effects in the 10 µg/ml of concentration (Table 2).

Extracts having anti-bacteria effect more than 40% were selected and repeated experiments to *S. aureus, E. coli* and *C. albicans*. The extracts of Euc had high anti-bacterial ability among the herbal extracts. Especially, stem (Euc-2) and flower (Euc-3) were effective, each 36 and 51% at 1000 µg/ml to *S. aureus.* Their anti-microbial effects were increased in dose-dependent manner. The anti-microbial effects were significantly increased from 100 µg/ml of the extracts. The Euc-2 and Euc-3 inhibited the growth of *E. coli* very weakly. Euc-2 did not show anti-microbial effect to *E. coli* (Figure 1). The extracts were diluted at 100, 500 and 1000 µg/ml and treated to *S. aureus, E. coli* and *C. albicans*. Their anti-microbial effects were measured by CFU. The results were similar to bacteria growth inhibition test. Colonies of the treated *S. aureus* were decreased in dose-dependent manner. Colonies of the treated *E. coli* were not decreased. (Figure 2). Effect of Euc-2 and Euc-3 to *C. albicans* has similar anti-fungal effect with treated to *S. aureus*, each 46 and 40% at 1000 µg/ml (Figure 3). Colonies of *C. albicans* were decreased in dose-dependent manner (Figure 4). Euc-3 was more effective to inhibit the replication of bacteria, whereas Euc-2 was more effective to inhibit the replication of fungi.

Table 2. Anti-bacterial effects of medicinal herb extracts and natural materials.

Sample name	Concentration (µg/ml)		
	10	100	1000
Cec-1	6.2	8.8	21.3
Cec-2	17.9	17.2	1.9
Cec-3	27.1	23.1	29.9
Cec-4	25.7	23.8	29.7
Y-Cec-1	0.3	7.9	13.2
Y-Cec-2	-3.1	6.6	16.1
Y-Cec-3	-10.3	6.0	13.4
Euc-1	8.6	11.6	18.4
Euc-2	4.3	6.6	**40.8***
Euc-3	16.5	19.1	**52.4***
R-Euc-1	-10.2	3.3	4.6
R-Euc-2	-1.8	2.9	23.1
R-Euc-3	4.7	10.8	11.8
Evp-1	18.7	28.5	34.0
Evp-2	13.8	24.9	-25.4
Evp-3	11.8	19.6	-29.4
Ang-1	19.1	32.4	19.2
Ang-2	18.2	12.5	2.9
Ang-3	17.9	10.9	13.3
Cii-1	23.6	26.3	34.2
Cii-2	19.7	10.1	0.6
Aba-1	4.1	7.8	19.6
Aba-2	10.7	17.4	9.1
Aba-3	16.7	8.6	2.2
Gog-1	9.5	11.3	24.0
Gog-2	10.1	15.8	29.6
Gog-3	0.4	9.4	17.5
Brc-1	18.3	20.0	21.2
Brc-2	15.6	10.6	20.7
Fulvic acid	17.0	15.0	21.0
Humic acid	16.0	13.0	21.0
Quercetin	3.1	5.8	4.6
Phloroglucinol	2.0	4.0	4.0

*More than 40% anti-bacterial effect.

Anti-oxidant effects of extracts of medicinal herbs

DPPH radical scavenging test were conducted to confirm an anti-oxidant effect of twenty nine medicinal herbs. Evp showed the highest anti-oxidant effects among the used extracts (Table 3).

Extracts having anti-oxidant effect more than 70% were selected and tested through three independent experiments. Quercetin (78%), phloroglucinol (71%), Evp-2

Table 3. Anti-oxidant effects of medicinal herb extracts and natural materials by DPPH radical scavenging test.

Sample name	Concentration (µg/ml)	
	100	1000
Cec-1	10.0	17.9
Cec-2	5.0	0.0
Cec-3	4.5	3.8
Cec-4	4.5	9.8
Y-Cec-1	0.0	7.6
Y-Cec-2	0.0	8.1
Y-Cec-3	25.3	24.4
Euc-1	3.8	12.1
Euc-2	11.5	20.4
Euc-3	1.3	12.6
R-Euc-1	3.8	9.3
R-Euc-2	13.3	19.9
R-Euc-3	4.8	12.6
Evp-1	7.3	24.7
Evp-2	16.0	**73.8***
Evp-3	30.0	**87.9***
Ang-1	10.8	21.9
Ang-2	12.5	21.9
Ang-3	6.8	19.1
Cii-1	3.3	4.0
Cii-2	3.3	4.5
Aba-1	0.2	19.4
Aba-2	4.3	19.1
Aba-3	4.5	10.0
Gog-1	12.0	1.0
Gog-2	10.8	16.4
Gog-3	4.8	6.8
Brc-1	1.3	2.3
Brc-2	11.5	19.4
Fulvic acid	24.0	37.8
Humic acid	11.5	15.7
Quercetin	30.0	**88.4***
Phloroglucinol	50.0	**80.9***

*More than 70% radical scavenging effect.

Table 4. Cholesterol adhesion ability of medicinal herb extracts and natural materials in 10 mg/ml.

Sample name	Cholesterol adhesion ability (%)
Cec-1	7.8
Cec-2	-23.7
Cec-3	**43.1***
Cec-4	-13.4
Y-Cec-1	-40.9
Y-Cec-2	-13.4
Y-Cec-3	-22.4
Euc-1	-17.2
Euc-2	-9.5
Euc-3	-13.8
R-Euc-1	6.9
R-Euc-2	-30.6
R-Euc-3	-7.8
Evp-1	-39.2
Evp-2	-10.8
Evp-3	8.6
Ang-1	-13.8
Ang-2	**21.1***
Ang-3	12.9
Cii-1	4.3
Cii-2	3.9
Aba-1	4.3
Aba-2	-38.4
Aba-3	4.7
Gog-1	4.7
Gog-2	0.0
Gog-3	-19.8
Brc-1	2.6
Brc-2	2.6
Fulvic acid	**51.3***
Humic acid	58.2*
Quercetin	46.4*
Phloroglucinol	59.1*

*More than 20% cholesterol adhesion ability.

(70%) and Evp-3 (82%) showed high anti-oxidant effects. Evp-2 (stem) and Evp-3 (flower) had similar ability to quercetin and phloroglucinol which are strong anti-oxidants (Figure 5). Evp-3 is more effective than Evp-2.

Cell protection effect of *Oenothera odorata*

To confirm an anti-oxidant effect in cell using MTT assay, cell protection of the extracts against oxidative stress induced by H_2O_2 were tested. While quercetin and Evp-2 were effective at concentration no less than 1000 µg/ml, phloroglucinol and Evp-3 were effective at concentration no less than 500 µg/ml. Evp-3 is more effective than Evp-2 (Figure 6).

Anti-cholesterol effects of medicinal herbs

Anti-cholesterol effects of the extracts were tested by cholesterol adhesion test. Cholesterol adhesion abilities of the extracts were observed clearly. Fulvic acid, humic acid, phloroglucinol and quercetin have high cholesterol adhesion ability greater than 40%. Cholesterol adhesion abilities of fulvic acid, humic acid, phloroglucinol and quercetin were 51.3, 58.2, 46.4 and 59.1% in concentration of 1 mg/ml, respectively. Adhesion ability of natural herb extracts were not much higher than positve control used in this study. However, Ang-2 which is stem of *Anethum graveolens* and Cec-3 which is flower of Red *Celosia cristata* were quite high among the extracts (21.1%, 43.1%, respectively) (Table 4). The cholesterol

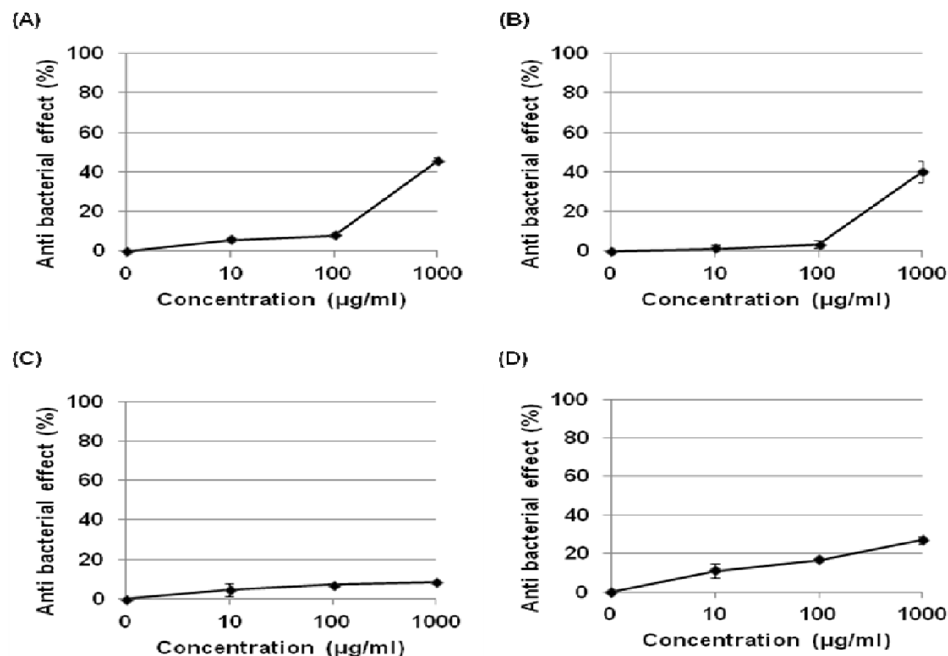

Figure 1. Inhibition of bacteria growth by Euc extracts. (A), Growth inhibition of *S. aureus* by Euc-2; (B), Growth inhibition of *S. aureus* by Euc-3; (C), Growth inhibition of *E. coli* by Euc-2; (D), Growth inhibition of *E. coli* by Euc-3.
These results were obtained by three independent experiments.

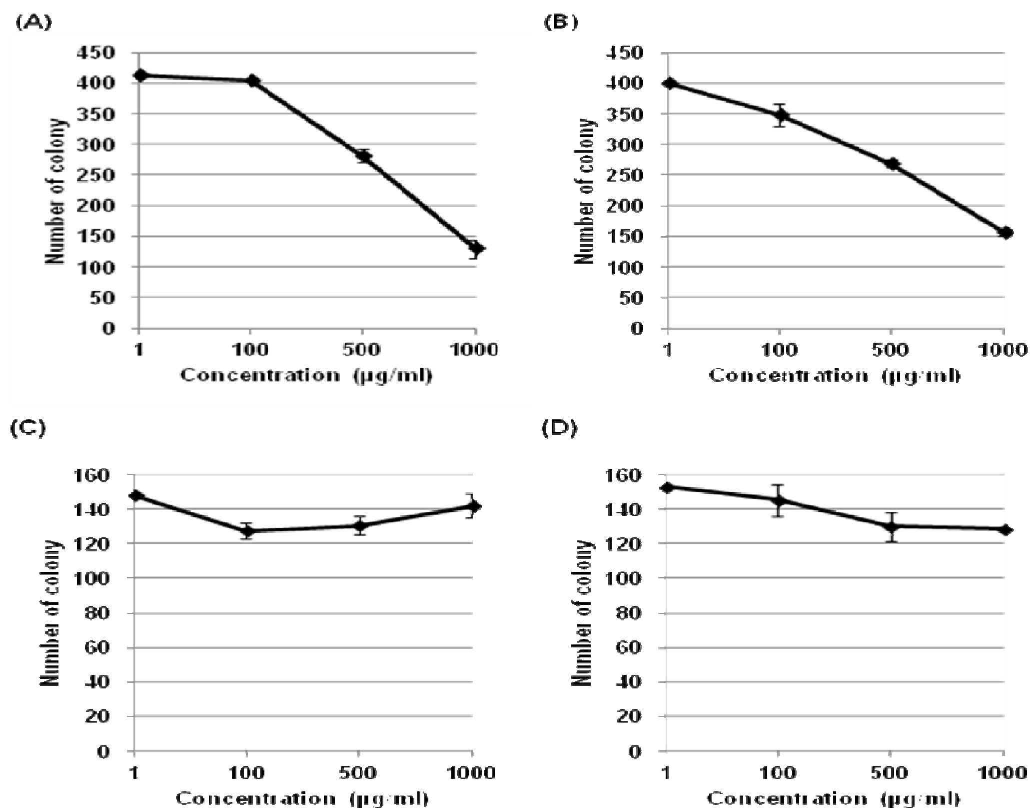

Figure 2. Anti-bacterial effects of Euc extracts by CFU. (A), Growth inhibition of *S. aureus* by Euc-2; (B), Growth inhibition of *S. aureus* by Euc-3; (C), Growth inhibition of *E. coli* by Euc-2; (D), Growth inhibition of *E. coli* by Euc-3.
These results were obtained by three independent experiments.

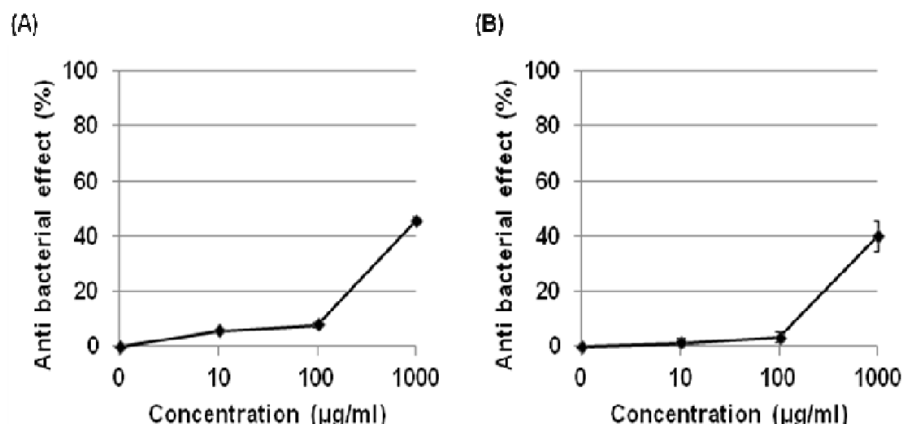

Figure 3. Inhibition of fungus growth of Euc extracts. (A), Growth inhibition of *C. albicans* by Euc-2; (B), Growth inhibition of *C. albicans* by Euc-3. These results were obtained by three independent experiments.

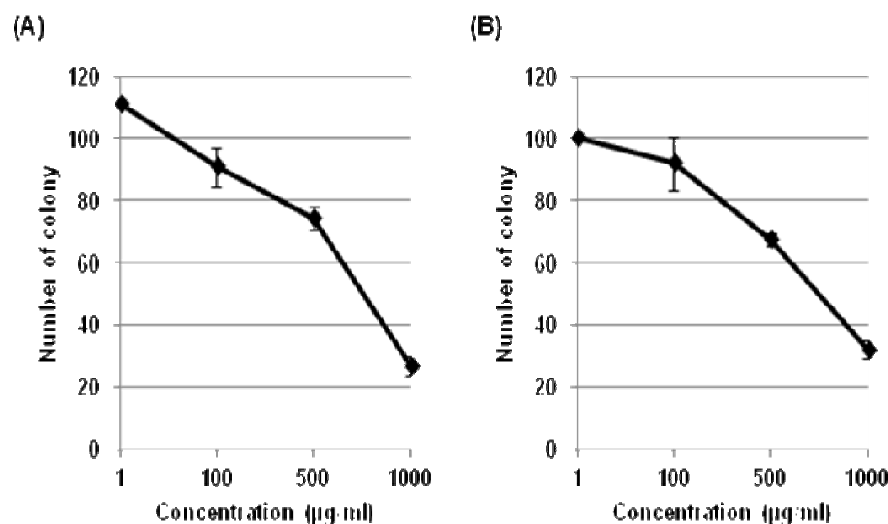

Figure 4. Anti-fungal effects of Euc extracts by CFU. (A), Growth inhibition of *C. albicans* by Euc-2; (B), Growth inhibition of *C. albicans* by Euc-3. These results were obtained by three independent experiments.

adhesion ability of fulvic acid was highest among all materials in low concentration (100 µg/ml), followed by Cec-3, humic acid, quercetin, phloroglucinol and Ang-2 in order (Figure 7).

Inhibition effects of HCV genotype 2a replication by the functional medicinal herbs

In order to elucidate the inhibition effects of HCV genotype 2a RNA replication, the extracts were treated in HCV2a cell. Phloroglucinol and Evp-2 slightly inhibited HCV RNA replication. Humic acid, quercetin and Cec-3 were markedly inhibited HCV RNA replication. Especially, fulvic acid completely inhibited HCV RNA replication (Figure 8). This result indicated that inhibition of HCV RNA replication is dependent on reduction of cholesterol (Figure 7 and 8).

DISCUSSION

There are many studies of functional effect such as anti-bacteria, anti-oxidant and anti-allergy of medicinal herbs and natural materials. However, the studies of anti-viral effect are few. In most studies, they used natural extracts thoughtlessly and prooflessly. In this study, we focused anti-viral effect according to the functional effect like anti-bacterial, anti-oxidant and anti-cholesterol effect. As a result, natural extracts having anti-bacterial and anti-oxidant effect were not related with inhibition of HCV genotype 2a RNA replication.

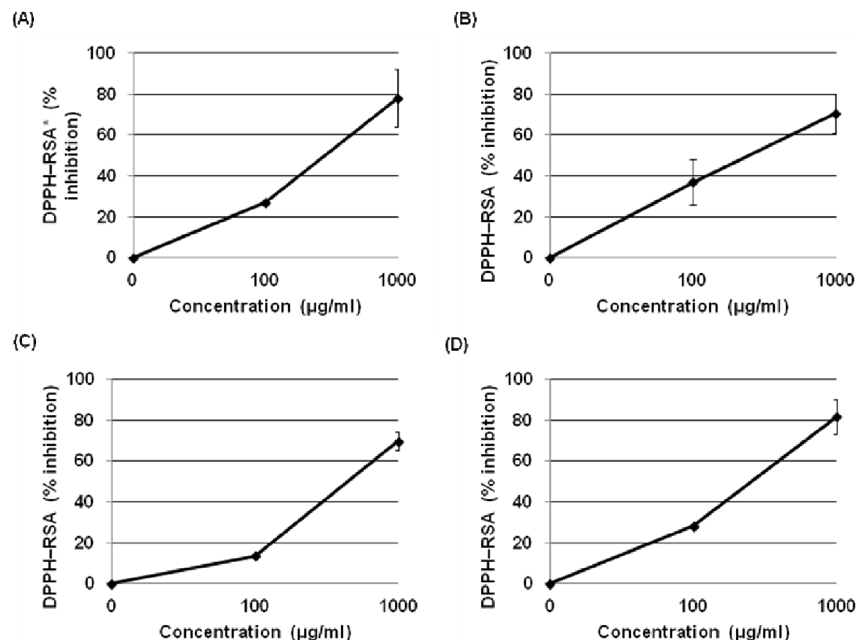

Figure 5. DPPH radical scavenging activities of Evp extracts (A), Quercetin; (B), Phloroglucinol; (C), Evp-2; (D), Evp-3. * RSA ; radical scavenging assay.
These results were obtained by three independent experiments.

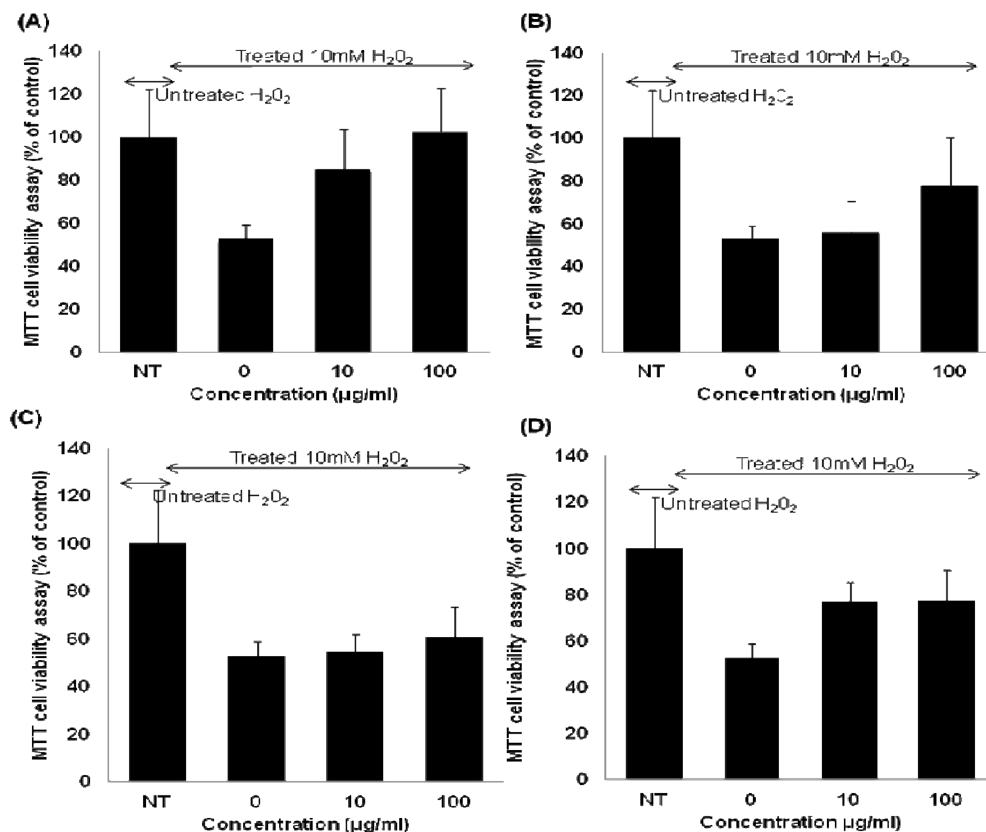

Figure 6. Cell protection abilities of Evp extracts against oxidative stress by MTT assay (A), Quercetin; (B), Phloroglucinol; (C), Evp-2; (D), Evp-3. *Treat, They were treated with 10mM H_2O_2; Untreat, They were not treated with 10mM H_2O_2. These results were obtained by three independent experiments.

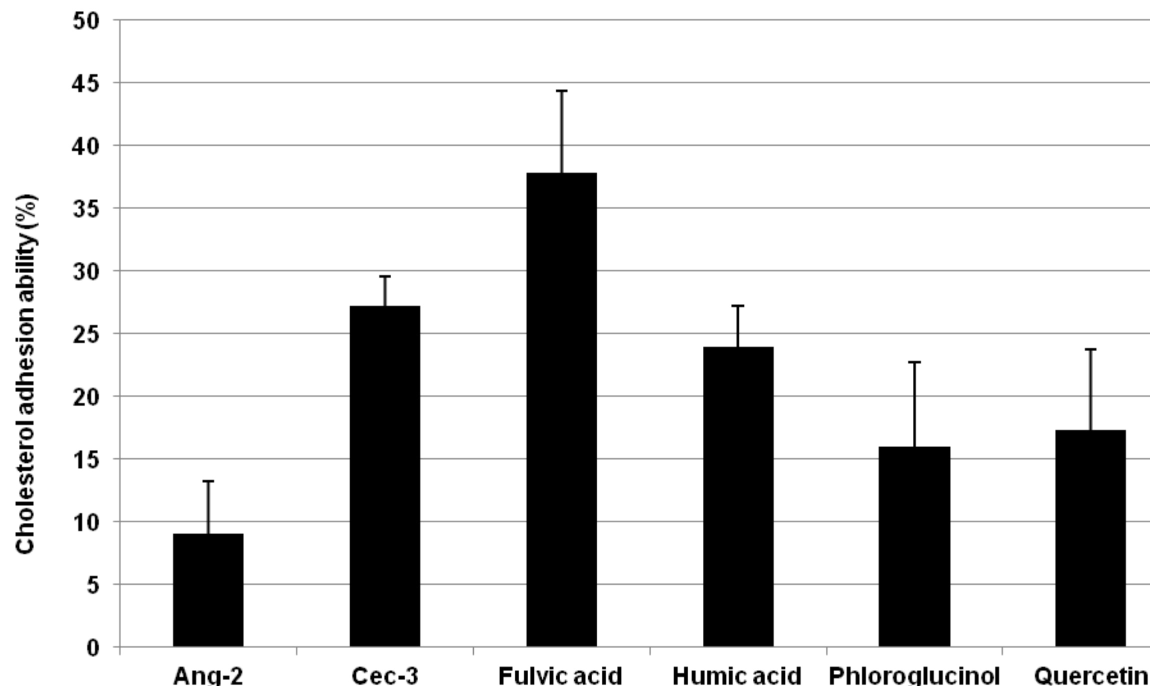

Figure 7. Cholesterol adhesion abilities of the selected medicinal herbs and natural materials in 100 µg/ml These results were obtained by three independent experiments.

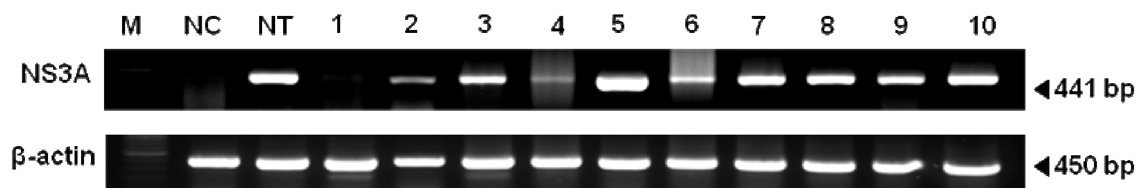

Figure 8. Suppression of HCV2a replication by the functional medicinal herb extracts M : marker, NC : Huh7 cell, NT : non treated to HCV 2a cell, 1 : Fulvic acid (100 µg/ml), 2 : Humic acid (100 µg/ml), 3 : Phloroglucinol (100 µg/ml), 4 : Quercetin (10 µg/ml), 5 : Ang-2 (100 µg/ml), 6 : Cec-3 (100 µg/ml), 7 : Euc-2 (10 µg/ml), 8 : Euc-3 (10 µg/ml), 9 : Evp-2 (10 µg/ml), 10 : Evp-3 (10 µg/ml).

Otherwise, anti-cholesterol effect has correlation with anti-HCV effect. It corresponds with other studies that cholesterol has relation to HCV proliferation (Kapadia et al., 2005; Kapadia et al., 2007; Roe et al., 2011; Shavinskay, 2007). Nevertheless, it does not coincide perfectly. Although Ang-2, Cec-3, fulvic acid, humic acid, phlorogucinol and quercetin have cholesterol adhesion ability, Ang-2 does not have an anti HCV effect because it showed weak cholesterol reduction effect. Moreover, anti-HCV ability of phloroglucinol which have the greatest cholesterol adhesion ability was not high. However, cholesterol adhesion ability in low dose was corresponded with anti-viral effect. We anticipated the reason why the 100 µg/ml was the concentration treating to cell. We need more experiments about anti-cholesterol effect in cell.

Anti-HCV effect is different by HCV genotype. For example, HCV genotype 1b is more sensitive to HMG-CoA reductase inhibitors than HCV genotype 2a (Chang and Jo, 2011). So, we expect that medicinal herb extracts are more effective to inhibition of HCV genotype 1b replication.

When treating natural extracts to cell, cell toxicity is problem to experiments. Especially, extracts of *Eupatorium chinensis*. spp had a lot of cytotoxicity (data not shown). Study of their cytotocicity should be needs to establish whether the extracts have cytotoxicity or anti-cancer effect. As a result of anti-oxidant effect, stem and flower extracts of *Oenothera odorata* had effect as high as positive controls such as quercetin and phloroglucinol. If a single component having anti-oxidant effect is isolated from *O. odorata,* it would be a strong an antioxidant

component. Likewise, natural herb extracts had lower effects than single components mostly in experiment.

There are a few papers which showed anti-HCV effect using natural extracts. Anti-viral effect of *Acacia nilotica* and *Solanum nigrum* against HCV were also established (Javed et al., 2011, Rehman et al, 2011b). *S. nigrum* have twelve percent of cholesterol adhesion ability in this study (data not shown). Therefore, we expect that Ang-2, Cec-3, fulvic acid, humic acid, phlorogucinol and quercetin having more cholesterol adhesion ability than *S. nigrum* are more effective to inhibit HCV replication.

Celosia cristata have been used to treat diarrhea and uteritis as herbal medicine. Saponins are abundant in seed of *C. cristata*. The seed of them have anti-inflammatory and anti-tumor effects (Wu et al., 2011).

Though there are a few reports about effect of seed extracts of *C. cristata*, there is no report about effects of the flower extracts. This is first report about anti-cholesterol and anti-HCV effects of *C. cristata.* We expect another component except for saponin show these effects. The component should be isolated and studied more to elucidate mechanisms of correlation between cholesterol reduction and suppression of HCV replication. Therefore, we expect development of anti-HCV drug using the natural extracts.

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