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Antiviral activity of herbal extracts against the hepatitis A virus

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A B S T R A C T

Herbal plants have long been used as traditional medicines to treat diseases caused by microbial pathogens. The hepatitis A virus (HAV) causes acute liver infection through the fecal–oral route. Although the antimicrobial activities of herbal extracts against bacterial and some viral pathogens have been extensively studied, their antiviral properties against HAV have not been investigated thus far. This study was designed to investigate the inhibitory effect of 16 herbal extracts against HAV. Significant inhibition of HAV was observed only when HAV was co-treated with extracts. Ten out of the 16 herbal extracts demonstrated significant virucidal activity against HAV. *Alnus japonica* extract at a concentration of 50 μg/mL reduced HAV titer by 3.43 ± 0.24 logs. *Artemisia annua, Allium sativum, Allium fistulosum,* and *Agrimonia pilosa* extracts showed 2.33 ± 0.43, 2.10 ± 0.41, 2.07 ± 0.60, and 2.03 ± 0.26-log reductions, respectively. *Pleuropterus multiflorus, Eleutherococcus senticosus, Coriandrum sativum, Ginkgo biloba,* and *Torilis japonica* extracts reduced HAV titer by 1.02 ± 0.21 to 1.90 ± 0.33 logs. Among the 10 herbal extracts, *Alnus japonica* extract was the most potent in inhibiting HAV without exhibiting cytotoxicity.
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1. Introduction

Since an Indian medical book first described 600 herbal plants in 1000 B.C., various herbs have been used in oriental medicine to treat diseases from about 3000 years ago (Singh, 2011). Herbal extracts contain multiple secondary metabolites such as terpenoids, saponins, polyphenols, alkaloids, and flavonoids (Wink, 2015). They are able to structurally interact with the cell membrane, proteins, and DNA bases. Furthermore, they potentially act alone or synergistically against infection by various bacteria, fungi, parasites, and viruses in humans (Wink, 2015). In Europe, some traditional medicines have been developed into modern drugs through clinical trials (Wink, 2015).

Herbal extracts are well known to have antimicrobial, antioxidant, antiinflammatory, anticancer, and antidiabetic activities (Atta & Alkofahi, 1998; Cai, Luo, Sun, & Corke, 2004; Dorman & Deans, 2000; Patel, Kumar, Laloo, & Hemalatha, 2012). In particular, *Agrimonia pilosa, Allium sativum, Allium fistulosum, Alnus japonica, Artemisia annua, Coriandrum sativum, Eleutherococcus senticosus, Ginkgo biloba, Pleuropterus multiflorus,* and *Torilis japonica* were reported to have antiviral properties against enveloped or non-enveloped viruses (Asres et al., 2001; Efferth et al., 2002; Glatthaar-Saalmüller, Sacher, & Esperester, 2001; Haruyama & Nagata, 2013; Kim et al., 2010; Lee et al., 2012; Ma et al., 2002; Shin, Lee, Park, & Seong, 2010; Tung et al., 2010; Weber et al., 1992) (Table 1). Although the antimicrobial activities of herbal extracts against bacterial and some viral pathogens have been extensively studied (Ding, Liao, Huang, Zhou, & Chen, 2006; Nascimento, Locatelli, Freitas, & Silva, 2000), studies investigating the antiviral property of herbal extracts against foodborne viruses are limited.

The hepatitis A virus (HAV) is a single-stranded, non-enveloped, positive-sense RNA virus belonging to the family Picornaviridae (Fiore, 2004). Acute hepatitis caused by HAV is still a public health problem worldwide (Fiore, 2004). Since HAV and enteric viruses are highly resistant to environmental conditions such as ambient temperature and low pH, they can persist for up to 2 months on environmental surfaces (Kramer, Schwebke, & Kampf, 2006). Although resistance to disinfectants was reported for viruses belonging to the Picornaviridae family, chlorine has been demonstrated to be successful in reducing the titer of resistant viruses in produce and water. However, such chemical disinfectants are associated with the formation of toxic by-products (Martin et al., 2013).

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Table 1
Species, common names, and sources of herbal plants used in this study and their target viruses described in previous publications.

Genus and species	Common names	Source	Type of virus	Target viruses	References
Alnus japonica	Japanese alder	Leaf	Enveloped RNA virus	Influenza virus	(Tung et al., 2010)
Ginkgo biloba	Maidenhair tree	Leaf		Influenza virus	(Haruyama & Nagata, 2013)
Agrimonia pilosa	Maidenhair tree	Whole plant		Influenza virus	(Shin et al., 2010)
Allium fistulosum	Welsh onion	Whole plant		Influenza virus	(Lee et al., 2012)
Pleuropterus multiflorus	Chinese knotweed	Whole plant		Respiratory syncytial virus	(Ma et al., 2002)
Paeonia lactiflora	Chinese peony	Whole plant		Respiratory syncytial virus	(Lin, Wang, Lin, Chiang, & Chang, 2013)
Coriandrum sativum	Chinese parsley	Whole plant		Human immunodeficiency virus	(Asres et al., 2001)
Eucommia ulmoides	Gutta-percha tree	Stem bark		Human immunodeficiency virus	(Lv et al., 2008)
Torilis japonica	Erect hedgeparsley	Whole plant		Mouse hepatitis virus	(Kim et al., 2010)
Cornus officinalis	Dogwood	Fruit	Non-enveloped RNA virus	Coxsackievirus	(Song et al., 2015)
Eleutherococcus senticosus	Siberian ginseng	Leaf and stem	Enveloped RNA virus Non-enveloped RNA virus	Human rhinovirus, respiratory syncytial virus, and influenza A virus	(Glatthaar-Saalmüller et al., 2001)
Sophora flavescens	Shrubby sophora	Root	Enveloped DNA virus	Hepatitis B virus	(Ding et al., 2006)
Artemisia annua	Sweet wormwood	Leaf		Human cytomegalovirus and herpes simplex virus type 1	(Efferth et al., 2002; Karamoddini, 2011)
Allium species (*Allium thunbergii*)	Chinese chives	Whole plant	Non-enveloped DNA virus	Adenovirus	(Chen et al., 2011)
Vitis vinifera	Wild grape	Fruit	Enveloped RNA virus Enveloped DNA virus	Herpes simplex virus type-1 Parainfluenza viruses	(Orhan, Orhan, Ozcelik, & Ergun, 2009)
Allium sativum	Garlic	Whole plant	Enveloped RNA virus Non-enveloped RNA virus Enveloped DNA virus	Herpes simplex virus type 1 and 2, parainfluenza virus type 3, vesicular stomatitis virus, vaccinia virus, and human rhinovirus type 2	(Weber et al., 1992)

Outbreaks of HAV infection mainly occur through ingestion of contaminated food such as salads, sandwiches, frozen raspberries, frozen strawberries, iceberg lettuce, green onions, shellfish, and raw beef (Fiore, 2004). Since it is very difficult to detect the contamination of food or environments by HAV, a diversified approach to prevent the contamination or infection by HAV has been developed (Fiore, 2004). The co-treatment with grape seed extract has been applied to decontaminate produce from viral and bacterial pathogens (Su & D'Souza, 2011). In addition, pre-treatment using Korean red ginseng (KRG) extract or ginsenosides reduced the HAV titer in fetal rhesus monkey kidney cells (FRhK-4) (Lee, Lee, Lee, & Choi, 2013). The application of plant extracts against enteric viruses has been considered an alternative to chemical agents in food safety (Perumalla & Hettiarachchy, 2011).

The aim of this study was to compare the inhibitory effect of pre-treatment and co-treatment of herbal extracts against HAV on FRhK-4 cells, and to select the most potent *anti*-HAV candidate.

2. Materials and methods

2.1. Virus and cell lines

Fetal rhesus monkey kidney (FRhK-4) (ATCC, Manassas, VA, USA) cells were cultured in Dulbecco's modified Eagle medium (DMEM; Gibco-Invitrogen, Grand Island, NY, USA) supplemented with sodium bicarbonate, 10% fetal bovine serum (Gibco-Invitrogen), and 1% antibiotics-antimycotics (Gibco-Invitrogen). The FRhK-4 cells were infected with HAV HM-175 strain (ATCC) and incubated for 5 d to propagate the virus. The cultured virus was freeze-thawed for 3 cycles and centrifuged at 2500g for 15 min.

2.2. Herbal extracts

Sixteen herbal extracts were purchased from the Korea Plant Extract Bank at the Korean Research Institute of Bioscience & Biotechnology (KRIBB; Cheongwon, Korea) (Table 1). All 16 plant extracts were prepared using 99.9% methyl alcohol (HPLC grade) at 45 °C. Briefly, plants were precipitated for 2 h following sonication for 15 min at 45 °C, which was repeated for 3 d (SD-Ultrasonic Cleaner, SDN-900H). Subsequently, extracts were concentrated using an evaporator at 45 °C (EYELA Rotary Evaporator, N-1000SWD). Concentrated extracts were dried for 24 h at −70 °C and stored at −4 °C (Biotron Corporation, Modul Spin 40). All herbal extracts were dissolved in dimethyl sulfoxide (DMSO), diluted with sterile distilled water, and sequentially filtered using syringe filters with pore sizes of 5, 1.2, 0.8, 0.45, and 0.20 μm. The pH of each extract was measured by a pH meter (Beckman Coulter, Fullerton, CA, USA).

2.3. Cell viability assay

The cytotoxicity of the 16 herbal extracts was assessed by use of a cell counting kit 8 (CCK-8) (Sigma-Aldrich). FRhK-4 cells were seeded in a 96-well plate at a density of 5000 cells/well and cultured at 37 °C, 5% CO_2 for 24 h. Cells were then treated with each herbal extract at a concentration of 10, 50, and 100 μg/mL. The 96-well plate was incubated in a CO_2 incubator for 24 h, and then 10 μL of CCK-8 solution was added to each well of the plate. Four hours later, the absorbance value was read at a wavelength of 450 nm using an Epoch spectrophotometer (Biotek, Winooski, VT, USA). The cell viability percentage was calculated as follows:

$$\text{Cell viability (\%)} = (OD_{test} - OD_{blank})/(OD_{control} - OD_{blank}) \times 100$$

Where OD_{test} is the absorbance of the herbal extracts in CCK-8–treated cells, $OD_{control}$ is the absorbance of the medium and CCK-8 alone (no cells), and OD_{blank} is the absorbance of solvent blanks (sterile distilled water and DMSO).

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