

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Antiviral Natural Products against Hepatitis-A Virus

*Damian Chukwu Odimegwu
and Uzochukwu Gospel Ukachukwu*

Abstract

The review on antiviral anti-hepatitis A virus agents is warranted given the importance of hepatitis A virus (HAV) as a human pathogen. Novel antiviral drugs have been sourced from natural agents and developed into products for management of viral infections. The role of purified natural products in treatment and as adjunctives in the management of HAV infections is clearly plausible. Treatments against Hepatitis A virus infection is currently limited. In this chapter, the antiviral natural products against hepatitis-A virus (HAV), their sources as well as their treatment approach and their application have been discussed. The antiviral natural products could be sourced generally from plants, herbs and animals. These natural agents have been shown to demonstrate substantial antiviral activity against HAV and could target various stages of the viral life cycle, replication, assemblage, release, as well as targeting virus-host specific interactions.

Keywords: hepatitis A, antiviral, natural products, infections

1. Introduction

The role of purified natural products in prophylaxis, palliative and curative treatment of myriad diseases of bacterial, fungal and viral origin cannot be overemphasized. Novel antiviral drugs have been sourced from natural agents and developed into products for prophylactic and therapeutic purposes [1]. These natural agents have been shown to demonstrate antiviral activity by interfering with viral life cycle, replication, assemblage, release, as well as targeting virus-host specific interactions [1]. Antiviral natural products can be sourced generally from plants or herbs, microbes, animals and humans. In this chapter, the antiviral natural products against hepatitis-A virus (HAV), their sources as well as their treatment approach and their application were adequately discussed.

2. Therapeutic anti-HAV natural products

Hepatitis A virus is among the pathogens that find their way into the human system through ingestion of food contaminated with them, and most of these food-borne viruses lack licensed antivirals. Vaccine development and immunization

against several viruses including hepatitis A virus lack preventive and efficient anti-viral therapies, as they are often challenged by counter-production of viral escape mutants that evade the immune system [1]. Also, the development of efficient and low-cost vaccines for economically unprivileged countries will be difficult, including countries with low prevalence where vaccine is recommended only for high-risk individuals [2]. Post-exposure of the human system to viral infections requires an efficient therapeutic approach to clear infections off the human system. It is imperative to develop effective antiviral therapeutic agents against these viruses, and interest in the employment of natural products as effective antiviral therapeutic agents has widely increased.

Flavonoids, polyphenols, saponin, proanthocyanins, polysaccharides, organic acids, proteins, polypeptides, and essential oils obtained from plant, animals or microorganisms can control and eradicate food-borne viral infections including hepatitis A [3, 4]. Over the past two decades, much effort has been aimed at identifying natural products, mostly of plant origin, to control food-borne viruses. Extracts from natural plants potentially have several applications, not limited to increasing the safety of food products and enhancing their quality, but also to serve as natural antiviral agents. For instance, these extracts possess several natural compounds that have been reported to demonstrate virucidal activity against surrogates of the human norovirus, a known food-borne virus [5]. In this section, we will discuss the antiviral therapeutic activities of several natural products and herbal medicines against hepatitis A viral infection.

2.1 Plant-based

2.1.1 Green tea extract

Green tea extract (GTE) is produced from the leaves of cultivated evergreen tea plant, *Camellia sinensis* L., of the family Theaceae [6]. It is rich in polyphenols and proanthocyanidins, and has been widely used to nutritionally enrich various food and beverages due to reports about its diverse health benefits such as possessing antioxidant, anti-inflammatory, and anti-carcinogenic properties [7–9]. Studies have revealed that GTE exhibits inhibitory properties against a wide variety of food-borne pathogens [10, 11]. Chemical composition of GTE includes mainly catechins, a group of flavonoids [12] that possess antimicrobial properties on a wide spectrum of Gram-positive and Gram-negative bacteria [11]. In a study, catechins such as epigallocatechin-3-gallate (EGCG) and epicatechin gallate (ECG), contained in GTE demonstrated the strongest antiviral properties [13], and also exhibited significant antiviral properties when encapsulated within chitosan electrosprayed microcapsules [14].

Recent *in vitro* study revealed that GTE demonstrated excellent antiviral activity against hepatitis A virus under controlled conditions of concentration, pH, temperature and also time exposure. It was shown that 5 mg/ml GTE incubated with the viral suspension for 2 h at 37°C and pH of 7.2 observed that there was complete inactivation of the virus in the suspension [6]. Findings suggested that GTE antiviral activity thrived better under increasing alkaline conditions. GTE has also been evaluated as a natural sanitizer of farm produce, demonstrating that HAV titers in lettuce and spinach were drastically reduced after 30 min treatment with 10 mg/ml GTE. Hence GTE holds promise for food-borne viral infection control through disinfection of food produce before consumption. Although the antiviral mechanisms of GTE have not yet been elucidated, some extrapolations could be drawn from the action of EGCG on viruses as it is the chief constituent compound in GTE [14, 15]. EGCG has high affinity for viral surface proteins but binds nonspecifically to them.

Therefore it exhibits its antiviral activity against a wide variety of enveloped and non-enveloped viruses by interfering with viral attachment to cell membrane receptors upon binding to them; thus, HAV infection could be curbed by GTE via similar mechanism.

2.1.2 Grape seed extract

Grape seed extract (GSE), *Vitis vinifera*, is generally obtained as a by-product of the grape juice and wine industry during processing of grapes [16]. It is reported to possess diverse bioactive principles including anthocyanins, flavonoids, proanthocyanidins, polyphenols, procyanidins and resveratrol, a derivative of stilbene [17]. The antioxidative, anti-inflammatory, cardioprotective, hepatoprotective, neuroprotective, and antimicrobial properties of these compounds make the extract to exhibit impressive pharmacological and therapeutic benefits [18].

GSE demonstrates antimicrobial activity against many food-borne bacterial pathogens including *Listeria monocytogenes*, *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), *Escherichia coli* O157:H7, *Salmonella enterica* serovar Enteritidis, and *S. typhimurium* [19–21]. Moreover, studies have reported the antiviral activities of GSE against some food-borne viruses including hepatitis A virus (HAV), human norovirus surrogates (feline calicivirus (FCV-F9)) and murine norovirus (MNV-1) [22, 23]. Under simulated gastrointestinal conditions, GSE reduced the HAV titer to undetectable levels in a dose-dependent fashion at varied temperatures (room temperature, 37°C) and time not exceeding 24 h. Emphatically, 2 mg/ml GSE drastically reduced HAV titer among other food-borne viruses to undetectable levels in intestinal fluid after 6 h.

However, this success may not be reproducible in the human system as the HAV strain, HM175, used during the study was a lab-adapted strain that was not sensitive to low pH as observed in the wild type strain. Again, some studies showed that GSE anti-HAV activity decreased in the presence of increasing concentrations of 0.02 and 0.2% dried milk or lettuce extract, where a higher dose is required to inactivate viral replication [24]. This implies that proteins could interfere with GSE antiviral activity and consequently decreases its effectiveness for treatments. Also, at concentrations ranging from 0.25 to 1 mg/ml GSE was said to diminish food-borne viral contamination levels on food produce (lettuce and peppers) without causing notable color changes on them. Therefore, GSE could be considered as a control measure for hepatitis A virus contamination on food produce before consumption, though may require a synergistic approach to combat persistent contamination of food produce.

The antiviral mechanisms of GSE are not yet well expounded. However, some studies suggest that resveratrol (RV), a nonflavonoid polyphenol found in grapes modulate some intracellular signaling pathways of the influenza virus [25]. In a study evaluating the effect of GSE on the adsorption and replicativity of HAV, it was revealed that treatment of the host cells with GSE prior to viral infection caused significant decline in HAV titer [26]. Post-viral infection of the host cells showed that HAV titers decreased insignificantly. This implies that GSE may have a moderate antiviral effect on adsorption of HAV on the host cells but with less effect on its replication [26]. Likewise, GSE was reported to down-regulate the expression of HIV entry coreceptors, implying that GSE may interrupt the binding of the virus to the cell receptors and in turn prevent HIV entry into normal lymphocytes [27]. Presently, GSE appears not to cause any structural damage to the viral capsid of HAV, rather it is more likely to exert greater antiviral activity by potentially blocking the host cell receptors and consequently prevents viral entry, replication, and infection.

2.1.3 Egyptian red sea seagrass extract

Seagrass is a critical part of the marine ecosystem and is generally distributed along the tropical and temperate coastal zones of the world [28]. It was said to be the only marine flowering plant that completes its lifecycle in sea water and often lives entirely submerged [29]. It is of ecological importance and is employed in folklore medicine for therapeutic purposes [30, 31]. The Egyptian Red Sea seagrass, *Thalassodendron ciliatum*, is said to be one of the longest and most common sea grasses along the Egyptian Red Sea. Its leaves are characterized by many 'tannin cells' more than in any other sea grass [32], which infers that it possesses a high phenolic content.

Compounds isolated from the sea grass crude extract have been shown to exhibit antioxidant and cytotoxic activities [28]. The crude extract demonstrated 100% inhibition of hepatitis A (HAV) and Herpes Simplex (HSV-1) viruses at 20 µg/mL. The antiviral activity of the crude extract against HAV was lost by fractionation, which could be explained by the synergistic action of several compounds in the crude extract [28]. Moreover, knowledge about the mechanism of anti-HAV activity of *T. ciliatum* has not yet been elucidated. Further studies are required to evaluate the toxicity of *T. ciliatum* on humans after consumption as food supplement or on formulation as a therapeutic drug against HAV.

2.1.4 Essential oils

Essential oils (EOs) are aromatic oily liquids derived from plant materials such as flowers, buds, seeds, leaves, branches, bark, grass, wood, fruit, and roots. Production of essential oils is majorly by steam distillation or by other methods such as solvent-heat extraction, pressing, fermentation or enfleurage [33]. Chemical components contained in these essential oils have been shown to be effective in combating pathogens [34, 35]. Few essential oils have been tested for their antiviral activities against food-borne viruses, particularly for HAV [36].

The anti-HAV activity of essential oils obtained from lemon (*Citrus limon*), sweet orange (*Citrus sinensis*), grapefruit (*Citrus paradisi*), and rosemary cineole (*Rosmarinus officinalis*) have been reported [33]. Essential oils belonging to the genus *Citrus* contain 85–99% of volatile compounds such as sesquiterpenes, monoterpene (limonene), and hydrocarbons, with their oxygenated products including aldehydes (citral), acids, ketones, alcohols (linalool), and esters [37]. *Rosmarinus officinalis* of the family, *Lamiaceae*, is generally applied during the preparation of some European cuisine and is also used as a medicinal plant, because of the strong antiseptic properties, antibacterial and antioxidant activities of its essential oil [38]; rosemary oil is also used as a natural food preservative [39, 40].

Essential oil treatment of ATCC/HM-175 strain of HAV propagated in Frp3 cells revealed that after an hour incubation at room temperature, the greatest reduction in cell infectivity was observed for rosemary cineole EO, followed by grapefruit and lemon EOs, while orange EO, although reducing HAV infectivity was not statistically significant [33]. Orange and grapefruit EOs were found to be cytotoxic for Frp3 cells at concentrations that exceeded 0.1%, while lemon and rosemary cineole EOs were cytotoxic at concentrations exceeding 0.5% and 0.05%, respectively. Studies have also revealed that treatment of contaminated berries with all four EOs from lemon, orange, grapefruit and rosemary cineole reduced the viral titer of HAV at room temperature. Essential oil from rosemary cineole was shown to be the most effective, as it significantly reduced the HAV titer on the berries followed by essential oils from grapefruit and lemon respectively [33]. Anti-HAV activity of essential oil from orange was not significant though there was a reduction in the

HAV titer on the berries. However, application of these essential oils alone may not be sufficient to decontaminate soft fruits (berries) laden with higher viral (HAV) loads [33]. Therefore, it is imperative that the essential oils be considered for use in food sterilization in combination with other treatments. It is also necessary to evaluate the minimum time it takes for EOs to reduce the maximum HAV loads on food produce so that adequate awareness is made to individuals to achieve food product safety before consumption [33]. Moreover, the mechanisms of anti-HAV activity of EOs have not yet been elucidated.

2.1.5 Korean red ginseng extract and ginsenosides

Ginseng (*Panax ginseng* Meyer) is a famous medicinal herb that has been used for over 5000 years in Korea and China [41]. Ginseng contains myriad bioactive components including, ginsenosides, phytosterols, polysaccharides, polyacetylenes, polyacetylenic alcohols, fatty acids and peptides [42]. There exists already documentations on the anti-stress, anti-carcinogenic, anti-inflammatory, antioxidant, anti-bacterial, anti-viral and anti-fungal activities of ginseng [42–44]. Furthermore, ginseng demonstrates useful activity on endocrine diseases, cardiovascular diseases and the immune system [45]. During processing, Red ginseng is usually steamed and fermented with skinned ginseng and this alters the composition saponin contained in it when done repeatedly [46]. Red ginseng has been shown to possess anti-cancer, anti-diabetic, anti-obesity and immunomodulatory properties [3, 4]. Likewise zidovudine, red ginseng has also been applied as a therapeutic supplement for the treatment of patients with human immunodeficiency virus [47].

Studies have shown that red ginseng extract and its ginsenosides inactivate food borne viruses such as the human norovirus (huNoV) surrogates (feline calicivirus and murine norovirus) [43]. A plaque assay performed on FRhK-4 cell lines pre-treated and co-treated with varied concentrations of Korean red ginseng (KRG) extract and purified ginsenosides (Rg1 and Rb1) showed that after inoculation of HAV HM-175 strain on the cell lines, KRG and the ginsenosides reduced significantly the HAV concentration [3, 4]. Korean red ginseng's extract demonstrated cytotoxicity at concentration above 10 µg/mL, while the purified ginsenosides showed no cytotoxic activity even up to 40 µg/mL. Although co-treatment of cell lines with KRG and the ginsenosides exhibited significant reduction of HAV concentration in the study, anti-HAV activity of the pretreated cell lines was quite higher [3, 4]. Hence, pretreatment with ginseng may be effective in preventing HAV infection. Also co-treatment of cell lines with KRG and the ginsenosides may be evaluated in further study using *in vivo* models.

The anti-HAV mechanisms of KRG extract and its ginsenosides are not clearly defined. However, reports from studies have shown that HAV-infected FRhK-4 cells activate the 2'-5' oligoadenylate synthetase/RNaseL pathway [48]. Activation of RNase L degrades viral RNA and cellular single-stranded RNA; hence, KRG extract and its ginsenosides may tour a similar path. In addition, previous studies have reported that ginseng polysaccharides and ginsenosides have the capacity to boost the production of cytokines via stimulation of immune cells [3, 4]. Interferons induced by this pathway also contribute to the antiviral response.

2.1.6 Blueberry juice and blueberry proanthocyanidins

Blueberries are said to contain about 88–261 mg of proanthocyanidin/100 g of edible portion according to the USDA database for flavonoid content (USDA Database for the proanthocyanidin Content of Selected Foods, August 2004). Again, blue berries possess some other structurally related polyphenols such as

anthocyanins and flavonoids [49]. Blueberry juice and its polyphenols have been found to have promising health benefits which include their cardioprotective, neuroprotective, anticarcinogenic, antibacterial, and antiviral properties [50]. Ethanol and water extracts of blueberries were reported to decrease *Listeria monocytogenes* by 5.90 log CFU/ml at 24 ppm and 37°C after 24 h *in-vitro* [51]. Also, 0.4 g/L gallic acid from blueberries caused a reduction in of *E. coli* O157:H7 titer in addition to the disruption of its cell-membrane after 24 h at 37°C *in-vitro* [52]. In addition, in a hepatitis C virus replicon cell system, methanol extract fraction of blueberry leaves (0.112–2200 lg/ml) was shown to suppress hepatitis C virus (HCV) subgenomic expression at 37°C after 72 h [53].

Recent study evaluated the antiviral activities of Blueberry juice and its proanthocyanidins (B-type) against HAV and some of human norovirus surrogates [50]. It was shown that in suspension, HAV titers were reduced by proanthocyanidins (2 and 5 mg/ml) to undetectable levels after 30 min, and after 3 h by 1 mg/ml proanthocyanidins. HAV titer was only reduced to by 2 log PFU/ml with Blue berry juice at pH 2.8 and 37°C after 24 h [50]. FRhK4 cells pre-infected and post-infected with HAV (strain; HM175) were also investigated for viral adsorption and replication upon treatment with the Blueberry juice and isolated proanthocyanidins [50]. The Blue berry proanthocyanidins showed promising preventive capacity as it moderately reduced HAV infectivity in the pre-infected cells but did not affect the replication of HAV in the post-infected cells. Hence, the Blue berry proanthocyanidins interrupt HAV binding and entry much more than it can limit its replication in the host cells; suggesting that it's antiviral efficacy is more preventive than therapeutic.

2.1.7 Aqueous extracts of *Hibiscus sabdariffa calyces*

Hibiscus sabdariffa, belonging to the family, Malvaceae, is an annual tropical or subtropical shrub species found in countries including Mexico, Sudan, India, and Thailand [54]. It is commonly called 'roselle' and is used for ornamental purposes, and the red calyces of *H. sabdariffa* are often used in the preparation of cold or hot beverages [55]. The calyces are said to be rich in bioactive compounds like anthocyanins, saponins, phenolic acids, organic acids and alkaloids [56]. Presence of organic acids like malic and tartaric acids identified in the calyces, possess a low pH of approximately 2–2.5 [54]. Aqueous extracts of the calyces are considered generally as safe and are approved for use as food additives by the U.S. Food and Drug Administration (21 CFR 172.510) in the flavoring of beverages [22, 23]. The calyces of *H. sabdariffa* are reported to possess a wide range of health benefits including antioxidant, anticancer, cardioprotective, anti-diabetic, and antimicrobial effects [57–59]. Protocatechuic acid (PCA), an essential component of *H. sabdariffa* has been shown to be the component responsible for its antimicrobial activity [60]. Another chemical component of the genus *Hibiscus*, known as Ferulic acid (FA) has also been reported to exhibit antimicrobial properties and antifilarial activity against *Setaria cervi* [61, 62].

Recent study evaluated the antiviral activity of *H. sabdariffa* against human novovirus surrogates and HAV. Findings revealed that aqueous extracts of calyces of *H. sabdariffa* (100 and 40 mg/ml) reduced HAV titer in suspension to undetectable levels at 37°C after 24 h [22, 23]. However, PCA demonstrated a moderate antiviral effect on HAV as it significantly reduced the HAV titer in suspension but not to undetectable levels. Pre- and post-infection assays with the aqueous extract of the calyces of *H. sabdariffa* (5 mg/ml) demonstrated no notable change in titres observed for HAV [22, 23]. Higher concentrations (40 and 100 mg/ml) of the aqueous extract was found to be cytotoxic to the host cell lines when added; observation for visual cytopathic effect under the light microscope showed that cells were

peeling off [22, 23]. It is likely the aqueous extract is effective for alleviating viral burden; however this has not yet been substantiated as more studies into model food systems and simulation of gastrointestinal tract conditions to test the efficacy of the extracts under *in vivo* conditions are required.

2.1.8 4-phenylcoumarin derivatives

Coumarin was first isolated from tonka beans, *Dipteryx odoranta*, also called Coumarou and biological activities of thousands of natural coumarins from plants, bacteria and fungi and chemical synthesis have been reported [63]. Coumarin and its derivatives have been used to manufacture drugs serving as anticoagulants including warfarin, acenocoumarin and phenprocoumon, and also for production of novobiocin, a potent inhibitor of bacterial DNA gyrase [63]. Coumarins (2H-chromen-2-ones) are recognized as a privileged bioactive scaffold for designing new agents with high affinity and specificity to various molecular targets [64], especially as antiviral agents [65]. In recent years, 4-Phenylcoumarins (neoflavones) which are bio-isosteres of flavonoids, have been of much interest as lead target structure for the discovery of new antiviral agents [66, 67].

A more recent study demonstrated that some coumarin derivatives possess anti-HAV activity. Newly modified 4-phenylcoumarin-based compounds were developed and evaluated for inhibition of 3C proteases [63]. Similar to other picornaviruses, HAV genome encodes a key processing protease, known as HAV 3C protease (HAV 3C^{pro}), which is a nonstructural cysteine protein responsible for the cleavage process within the viral polyprotein (250 kDa) that is critical for the replication process [63]. These proteases are responsible for processing the polyprotein precursor and also cleaving specific cellular factors needed for transcription and translation processes as well as nucleo-cytoplasmic trafficking in order to alter cell physiology to enhance viral replication; thus 3C^{pro} is vital to viral life cycle, making the viral 3C proteases choice targets for antiviral therapy [63]. Evaluation of the target compounds for their antiviral activity against hepatitis A virus revealed that the derivative, 1-(2-(2-Oxo-4-phenyl-2H-chromen-7-yloxy)acetyl) 4-ethylthiosemicarbazide had the most potent virucidal activity (IC₅₀ = 3.1 µg/ml, TI = 83). The derivatives, 2-(2-Oxo-4-phenyl-2H-chromen-7-yloxy)-N'-(1-(4-chlorophenyl)ethylidene)acetohydrazide and 2-(2-Oxo-4-phenyl-2H-chromen-7-yloxy)-N'-(1-(4-bromophenyl)ethylidene)acetohydrazide demonstrated the strongest virustatic effects against HAV adsorption and replication, respectively (IC₅₀ = 8.5 µg/ml, TI = 88; IC₅₀ = 10.7 µg/ml, TI = 91). Furthermore, studies reported that the three newly derived compounds were tested against HAV 3C protease and they exhibited remarkable inhibition effects (K_i = 1.903, 0.104 and 0.217 µM, respectively) indicating strong binding to HAV 3C^{pro} [63]. Also, the three compounds were docked within the pocket site of HAV 3C protease (PDB code: 2HAL) which illustrated that they had strong H-profiles with the amino acids Gly170 and Cys172. Findings suggested that the target compounds inhibited virus infection through the interrupting virus adsorption to the cell surface. This may have occurred via blocking of the cellular surface receptors by the target compounds which consequently led to an anti-HAV effect. Deduction from the post-treatment assay suggested that the target compounds inhibited the activities of some viral enzymes needed to complete the replication cycle or that they interfered with one or more steps in the viral life cycle.

2.1.9 Protamine, taxifolin and atropine

Protamine, a cationic peptide, is generally obtained from fish milt (spermatic cells) and is applied medically as a heparin antagonist, an injectable insulin-carrier,

and recently as an antibacterial ingredient in some food products [68]. Taxifolin (dihydroquercetin) is a flavononol amply found in grapes, olive oil, citrus fruits and onions [69]. It has been shown to possess strong pharmacological activities, including antioxidative, hepatoprotective, cardioprotective, anti-diabetic, anti-inflammatory, antitumor, neuroprotective effects, and had played a remarkable role in the preclusion of Alzheimer's disease [69]. Atropine is naturally occurring compound (alkaloid) majorly found in belladonna (Solanaceae) plants. It is a muscuranic receptor antagonist and is used medically to modulate muscular contractions and dilations which consequently regulate blood flow to cells and tissues [70].

A previous study investigated the inhibitory potential of protamine, atropine and taxifolin against HAV replication in PLC/PRF/5 cells, and found out that the trio exhibited some significant but not drastic effects on HAV replication [2]. Atropine demonstrated a concentration-dependent reduction in the infectivity of HAV but the antigenicity of the virus was not affected. HAV titer was reduced at the maximum concentration of 50, 59 and 50 µg/ml of protamine, taxifolin and atropine, respectively. It was suggested that further studies be done to determine the effect of these compounds on several multiplicities of HAV infection and also investigate possible synergistic effects of these compounds with other substances that have potential for clinical use against HAV infection [2]. The mechanisms of HAV titer reduction by the compounds are not yet clearly elucidated.

3. Adjunctive anti-HAV natural products

3.1 Japanese rice-koji miso extracts

Koji, also known as *Aspergillus oryzae*, is a filamentous fungus employed by the Japanese to ferment certain kinds of food like soybeans, potatoes, rice and some other grains [71]. Miso is one of the by-products of the fermentation of Japanese rice by Koji. Miso is conventional Japanese seasoning used for preparing miso soup, a staple Japanese cuisine [71]. Previous studies showed that Japanese miso extract increases the expression of a heat-shock protein known as glucose-regulated protein 78 (GRP78) and suppresses ultraviolet C mutagenesis [72]. Some researchers observed that HAV replication was retarded upon expression of GRP78 [71]; hence GRP78 has become a potential host antiviral against HAV infection [73]. Recent post-infection assay examined miso extracts obtained from Japanese rice-koji for antiviral activity against HAV, and it was shown that the miso extracts inhibited HAV replication by enhancing the expression of GRP78 in human hepatocytes (Huh7 and PXB cells) [71]. These findings suggested that Japanese miso extracts may synergistically work as antivirals against HAV infection by partially modulating GRP78 expression [71]. Miso extracts may also serve as effective dietary supplements for the control of acute hepatitis A infection.

3.2 Korean soy sauce

Conventional Korean soy sauce is generally made with germinated soybean, salt and water [74]. The soy sauce is fermented after cooking and crushing soybean, then mold it into a block form (Meju) with concurrent addition of salt (NaCl) and water before exposing it to natural conditions [3, 4]. The percentage salt content of traditional Korean soy sauce is around 16.3–20.8% NaCl [75]. Studies have shown that soy sauce possesses diverse biological activities such as angiotensin inhibitory, anti-platelet, anticarcinogenic, and anti-oxidant activities [74]. Also, there is a report about the antibacterial activity of soy sauce against *Escherichia coli* O157:H7 [76]. The

antimicrobial effects of soy sauce were attributed to the presence of a combination of ingredients and properties including NaCl, ethanol, pH, organic acids, and preservatives [74].

A study that evaluated the antiviral activity of the Korean soy sauce on HAV inoculated in raw fresh crabs (*Portunus trituberculatus*) to simulate storage conditions for homemade Ganjanggejang (a salted preserved raw seafood in Korean cuisine) revealed that there was an over 90% reduction of the HAV titer in the Ganjanggejang marinated in soy sauce containing 20% NaCl for at least 3 days [74]. Hence, the soy sauce was synergistically more effective at increasing salt concentrations. The antiviral activity of soy sauce is majorly due to the salt (NaCl) concentrations and partially attributable to its other constituents, such as ethanol, organic acids, and preservatives, and the pH of 5.11–6.98 [77]. Inhibition of HAV in crabs by NaCl in soy sauce might be due to changes in water activity which may affect virus survival [77]. In addition, antiviral mechanisms associated with NaCl may include altering the molecular structure of the viral RNA and inhibiting the viral enzymes' activity [74]. However, it's not likely that Korean soy sauce will be of relevance in clinical practice rather it may be instrumental for immediate food preservation and storage before consumption (Table 1).

Evaluated natural products	Concentration	Result	Proposed mechanism of action	References
Green Tea Extract	5 mg/ml for 2 h at 37°C and pH of 7.2	Complete inactivation of HAV in suspension	Interfers with viral attachment to cell membrane receptors upon binding to them	[7, 15]
Grape Seed Extract	2 mg/ml for 6 h at 37°C	Reduced HAV titer to undetectable levels under simulated gastrointestinal conditions	Interrupt the binding of HAV to the cell receptors, preventing adsorption.	[23, 24, 28]
Egyptian Red Sea Seagrass Crude Extract	20 µg/mL	100% inhibition of HAV in a plaque assay	—	[28]
Essential Oils (EO) from lemon, grapefruit and rosemary cineole	0.1% (EO from grapefruit); 0.5% (EO from lemon); 0.05% (EO from rosemary cineole)	Significant reduction in cell infectivity in the order; rosemary cineole > grapefruit > lemon.	—	[33]
Korean Red Ginseng Extract and Ginsenosides	5–10 µg/mL For 24 h at 37°C	Significant reduction of HAV titer with dose-dependent manner in pretreated FRhk-4 cells	(1) Activation of the 2'-5'oligoadenylate synthetase/RNaseL pathway; (2) boost the production of cytokines	[3–5, 49]
Blueberry Juice	pH 2.8 at 37°C for 24 h	Reduced HAV titer by 2 log PFU/ml	Interfers with HAV binding to host cells	[50]
Blueberry Proanthocyanidins	2 and 5 mg/ml for 30 min at 37°C	Reduced HAV titer to undetectable levels in suspension	Interrupt HAV binding and entry into host cells	[50]

Evaluated natural products	Concentration	Result	Proposed mechanism of action	References
Aqueous extracts of <i>Hibiscus sabdariffa</i> Calyces	100 mg/ml and 40 mg/ml at 37°C for 24 h	Reduced HAV titer to undetectable levels in suspension	—	[22, 23]
4-phenylcoumarin derivatives	10 µl at 37°C	Inhibited the activity of HAV 3C protease	Interrupt HAV adsorption on cell surface	[63]
Protamine	50 µg/ml	Reduced HAV infectivity	—	[2]
Taxifolin	59 µg/ml	Reduced HAV infectivity	—	[2]
Atropine	50 µg/ml	Reduced HAV infectivity	—	[2]
Japanese rice-koji miso extracts	—	Inhibited HAV replication	Inhibited HAV replication by enhancing the expression of GRP78 in human hepatocytes	[71]
Korean Soy Sauce	Containing 20% NaCl	over 90% reduction of the HAV titer	Inhibition of viral enzymes' activity	[74]

Table 1.
Summary of anti-HAV natural products.

4. Miscellaneous products

Duck hepatitis A virus type-1(DHAV-1) is a variant of hepatitis A virus that attacks ducks. It has been proposed that duck hepatitis A is a small animal model for the human hepatitis A [78]. It may be correct to say that antiviral agents against DHAV-1 will also demonstrate appropriate antiviral activity against human hepatitis A virus. Several natural agents have been under study to explore their antiviral potentials against DHAV-1 and they include phosphorylated *Codonopsis pilosula* polysaccharide (pCPP), Raw Rehmannia Radix Polysaccharide (RRRP), Baicalin phospholipid complex (BAPC), flavonoid combinations—baicalin-linarin-icariin-notoginsenosideR1 (BLIN).

It was reported that **RRRP** could significantly reduce mortality rate, liver lesion scoring, alleviate visual liver lesion, and decrease the alterations of plasma biochemical evaluation indexes of hepatic injury induced by DHAV-1 infection [79]. **pCPP** was also reported to demonstrate a strong inhibitory effect on DHAV-1 replication, which led to a significant decrease on the number of viral particles [80]. Studies with DHAV-1-infected ducklings treated with **BAPC** showed that it significantly inhibited DHAV-1 adsorption, replication and release [81]. Furthermore, it was reported that BAPC played anti-oxidative and immuno-supportive roles during the treatment, and that the immuno-supportive role was critical to the treatment. Another study evaluated the anti-DHAV-1 activity of a flavonoid mix, **BLIN** [82]. At 20 µg/mL, DHAV-1 inhibitory rate of BLIN at 20 µg/mL was reported to be 69.3% in duck embryonic hepatocytes. It was demonstrated that the survival rate of ducklings treated by BLIN was about 35.5%, which was remarkably higher than that of virus control (0.0%) [82]. In addition, after the treatment with BLIN, both

the hepatic injury and the oxidative stress of the infected ducklings assuaged [82]. Concurrently, a significant positive correlation was said to exist between the hepatic injury indices and the oxidative stress indices.

5. Future outlook

Currently, studies exploring potential anti-HAV natural products are still emerging and had attracted little attention, possibly because a vaccine has been developed to mitigate the spread of the viral infection to a considerable length of years. However, there is need for development of more efficient and effective anti-HAV therapeutic, prophylactic and adjunctive agents, and as at now, none has been licensed. Investigations into natural products with anti-HAV hold a promising outlook as several of them have demonstrated remarkable potential to control HAV infection and replication. In addition, studies should be aimed at mimicking more closely the features of the human hepatitis A virus *in vivo* than *in vitro* so as to clearly establish the basis for the application of these natural agents in a clinical setting. There is need to develop suitable animal models that could present very similar clinical manifestations as found in humans during hepatitis A virus infection, for more accurate interpretation and correlation of outcomes from pre-clinical studies involving natural products therapy. Hopefully, studies on antiviral natural products against HAV will gain ample attention in the nearest future.

Author details

Damian Chukwu Odimegwu^{1*} and Uzochukwu Gospel Ukachukwu²

¹ Faculty of Pharmaceutical Sciences, Department of Pharmaceutical Microbiology and Biotechnology, University of Nigeria, Nsukka, Enugu State, Nigeria

² Faculty of Biological Sciences, Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, Nigeria

*Address all correspondence to: damian.odimegwu@unn.edu.ng

IntechOpen

© 2020 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Lin L, Hsu W, Lin C. Antiviral natural products and herbal medicines. *Journal of Traditional and Complementary Medicine*. 2014;**4**(1):24-35
- [2] Biziagos E, Crance J-M, Passagot J, Deloince R. Effect of antiviral substances on hepatitis A virus replication in vitro. *Journal of Medical Virology*. 1987;**22**:57-66
- [3] Lee DY, Chung SJ, Kim KW. Sensory characteristics of different types of commercial soy sauce. *Journal of the Korean Society of Food Culture*. 2013;**28**:640-650
- [4] Lee MH, Lee B-H, Lee S, Choi C. Reduction of hepatitis a virus on frhk-4 cells treated with Korean red ginseng extract and ginsenosides. *Journal of Food Science*. 2013;**00**:M1-M4
- [5] Li D, Baert L, Uyttendaele M. Inactivation of food-borne viruses using natural biochemical substances. *Food Microbiology*. 2013;**35**:1-9
- [6] Randazzo W, Falcó-Ferrando I, Aznar R, Sánchez G. Effect of green tea extract on enteric viruses and its application as natural sanitizer. *Food Microbiology*. 2017;**66**:150-156. DOI: 10.1016/j.fm.2017.04.018
- [7] Cooper R, Morré DJ, Morré DM. Medicinal benefits of green tea: Part I. Review of non-cancer health benefits. *Journal of Alternative and Complementary Medicine*. 2005;**11**:521-528
- [8] Cooper R, Morré DJ, Morré DM. Medicinal benefits of green tea: Part II. Review of anticancer properties. *Journal of Alternative and Complementary Medicine*. 2005;**11**:639-652
- [9] Xia E-Q, Deng G-F, Guo Y-J, Li H-B. Biological activities of polyphenols from grapes. *International Journal of Molecular Sciences*. 2010;**11**:622-646
- [10] An B-J, Kwak J-H, Son J-H, Park J-M, Lee J-Y, Jo C, et al. Biological and anti-microbial activity of irradiated green tea polyphenols. *Food Chemistry*. 2004;**88**:549-555
- [11] Gadang V, Hettiarachchy N, Johnson M, Owens C. Evaluation of antibacterial activity of whey protein isolate coating incorporated with nisin, grape seed extract, malic acid, and EDTA on a turkey frankfurter system. *Journal of Food Science*. 2008;**73**:M389-M394
- [12] Yilmaz Y. Novel uses of catechins in foods. *Trends in Food Science & Technology*. 2006;**17**:64-71
- [13] Dhiman RK. The green tea polyphenol, epigallocatechin-3-gallate (EGCG)—One step forward in antiviral therapy against hepatitis C virus. *Journal of Clinical and Experimental Hepatology*. 2011;**1**:159-160
- [14] Gómez-Mascaraque LG, Sanchez G, López-Rubio A. Impact of molecular weight on the formation of electrosprayed chitosan microcapsules as delivery vehicles for bioactive compounds. *Carbohydrate Polymers*. 2016;**150**:121-130
- [15] Steinmann J, Buer J, Pietschmann T, Steinmann E. Anti-infective properties of epigallocatechin-3-gallate (EGCG), a component of green tea. *British Journal of Pharmacology*. 2013;**168**:1059-1073
- [16] D'Souza DH. Phytocompounds for the control of human enteric viruses. *Current Opinion in Virology*. 2014;**4**:44-49
- [17] Jayaprakasha GK, Selvi T, Sakariah KK. Antibacterial and antioxidant activities of grape

- (*Vitis vinifera*) seed extracts. Food Research International. 2003;**36**:117-122
- [18] Nassiri-Asl M, Hosseinzadeh H. Review of the pharmacological effects of *Vitis vinifera* (grape) and its bioactive compounds. Phytotherapy Research. 2009;**23**:1197-1204
- [19] Al-Habib A, Al-Saleh E, Safer AM, Afzal M. Bactericidal effect of grape seed extract on methicillin-resistant *Staphylococcus aureus* (MRSA). The Journal Toxicological Sciences. 2010;**35**:357-364
- [20] Kao TT, Tu HC, Chang WN, Chen BH, Shi YY, Chang TC, et al. Grape seed extract inhibits the growth and pathogenicity of *Staphylococcus aureus* by interfering with dihydrofolate reductase activity and folate-mediated one-carbon metabolism. International Journal of Food Microbiology. 2010;**141**:17-27
- [21] Perumalla AVS, Hettiarachchy NS. Green tea and grape seed extracts—Potential applications in food safety and quality. Food Research International. 2011;**44**:827-839
- [22] Joshi SS, Dice L, D'Souza DH. Aqueous extracts of *Hibiscus sabdariffa* calyces decrease hepatitis A virus and human norovirus surrogate titers. Food and Environmental Virology. 2015;**7**(4):366-373
- [23] Joshi SS, Su X, D'Souza DH. Antiviral effects of grape seed extract against feline calicivirus, murine norovirus, and hepatitis A virus in model food systems and under gastric conditions. Food Microbiology. 2015;**52**:1-10
- [24] Li D, Baert L, Zhang D, Xia M, Zhong W, Van Coillie E, et al. Effect of grape seed extract on human norovirus GII.4 and murine norovirus 1 in viral suspensions, on stainless steel discs, and in lettuce wash water. Applied and Environmental Microbiology. 2012;**78**:7572-7578
- [25] Palamara AT, Nencioni L, Aquilano K, De Chiara G, Hernandez L, Cozzolino F, et al. Inhibition of influenza A virus replication by resveratrol. The Journal of Infectious Diseases. 2005;**191**:1719-1729
- [26] Su X, D'Souza DH. Grape seed extract for the control of human enteric viruses. Applied and Environmental Microbiology. 2011;**77**:3982-3987
- [27] Nair MP, Kandaswami C, Mahajan S, Nair HN, Chawda R, Shanahan T, et al. Grape seed extract proanthocyanidins down regulate HIV1 entry co-receptors, CCR2b, CCR3 and CCR5 gene expression by normal peripheral blood mononuclear cells. Biological Research. 2002;**35**:421-431
- [28] Hamdy A-HA, Mettwally WSA, El Fotouh MA, Rodriguez B, El-Dewany AI, El-Toumy SAA, et al. Bioactive phenolic compounds from the Egyptian Red Sea seagrass *Thalassodendron ciliatum*. Zeitschrift für Naturforschung. 2012;**67c**:291-296
- [29] Short F, Carruthers T, Dennison W, Waycott M. Global seagrass distribution and diversity: A bioregional model. Journal of Experimental Marine Biology and Ecology. 2007;**350**:3-20
- [30] Hemminga MA, Duarte CM. Seagrass Ecology. Cambridge: Cambridge University Press; 2000. p. 298
- [31] Torre-Castro M, Rönnbäck P. Links between humans and sea grasses, an example from tropical East Africa. Ocean and Coastal Management. 2004;**47**:361-387
- [32] Lipkin Y. *Thalassodendretum ciliati* in Sinai (northern Red Sea) with special reference to quantitative aspects. Aquatic Botany. 1988;**31**:125-139

- [33] Battistini R, Rossini I, Ercolini C, Goria M, Callipo MR, Maurella C, et al. Antiviral activity of essential oils against hepatitis a virus in soft fruits. Food and Environmental Virology. 2019;**11**(1):90-95
- [34] Ozogul Y, Kuley E, Ucar Y, Ozogul F. Antimicrobial impacts of essential oils on food borne-pathogens. Recent Patents on Food, Nutrition & Agriculture. 2015;**7**(1):53-61
- [35] Piątkowska E, Rusiecka-Ziółkowska J. Influence of essential oils on infectious agents. Advances in Clinical and Experimental Medicine. 2016;**25**(5):989-995
- [36] Sánchez C, Aznar R, Sánchez G. The effect of carvacrol on enteric viruses. International Journal of Food Microbiology. 2015;**192**:72-76
- [37] Fisher K, Phillips C. Potential antimicrobial uses of essential oils in food: Is citrus the answer? Trends in Food Science & Technology. 2008;**19**(3):156-164
- [38] Nieto G. Biological activities of three essential oils of the Lamiaceae family. Medicines (Basel). 2017;**4**(3):63
- [39] Satyal P, Jones TH, Lopez EM, McFeeters RL, Ali NA, Mansi I, et al. Chemotypic characterization and biological activity of *Rosmarinus officinalis*. Food. 2017;**6**(3):20
- [40] Sirocchi V, Devlieghere F, Peelman N, Sagratini G, Maggi F, Vittori S, et al. Effect of *Rosmarinus officinalis* L. essential oil combined with different packaging conditions to extend the shelf life of refrigerated beef meat. Food Chemistry. 2017;**221**: 1069-1076
- [41] Yun TK. Brief introduction of *Panax ginseng* C.A. Meyer. Journal of Korean Medical Science. 2001;**16**:S3-S5
- [42] Gillis CN. *Panax ginseng* pharmacology: A nitric oxide link? Biochemical Pharmacology. 1997;**54**(1):1-8
- [43] Lee MH, Lee BH, Jung JY, Cheon DS, Kim KT, Choi C. Antiviral effect of Korean red ginseng extract and ginsenosides on murine norovirus and feline calicivirus as surrogates for human norovirus. Journal of Ginseng Research. 2011;**35**(4):429-435
- [44] Ng TB, Wang H. Panaxagin, a new protein from Chinese ginseng possesses anti-fungal, anti-viral, translation-inhibiting and ribonuclease activities. Life Sciences. 2001;**68**(7):739-749
- [45] Attele AS, Wu JA, Yuan CS. Ginseng pharmacology: Multiple constituents and multiple actions. Biochemical Pharmacology. 1999;**58**(11):1685-1693
- [46] Li H, Lee JH, Ha JM. Effective purification of ginsenosides from cultured wild ginsengroots, red ginseng, and white ginseng with macroporous resins. Journal of Microbiology and Biotechnology. 2008;**18**(11):1789-1791
- [47] Cho YK, Sung H, Lee HJ, Joo CH, Cho GJ. Long-term intake of Korean red ginseng in HIV-1-infected patients: Development of resistance mutation to zidovudine is delayed. International Immunopharmacology. 2001;**1**(7):1295-1305
- [48] Kulka M, Calvo MS, Ngo DT, Wales SQ, Goswami BB. Activation of the 2-5OAS/RNaseL pathway in CVB1 or HAV/18f infected FRhK-4 cells does not require induction of OAS1or OAS2 expression. Virology. 2009;**388**(1):169-184
- [49] Huang WY, Zhang HC, Liu WX, Li CY. Survey of antioxidant capacity and phenolic composition of blueberry, blackberry, and strawberry in Nanjing. Journal of Zhejiang University. Science. B. 2012;**13**:94-102

- [50] Joshi SS, Howell AB, D'Souza DH. Reduction of enteric viruses by blueberry juice and blueberry proanthocyanidins. Food and Environmental Virology. 2016;**8**(4):235-243
- [51] Park YJ, Biswas R, Phillips RD, Chen J. Antibacterial activities of blueberry and muscadine phenolic extracts. Journal of Food Science. 2011;**76**:M101-M105
- [52] Lacombe A, Tadepalli S, Hwang CA, Wu VC. Phytochemicals in low bush wild blueberry inactivate *Escherichia coli* O157:H7 by damaging its cell membrane. Foodborne Pathogens and Disease. 2013;**10**:944-950
- [53] Takeshita M, Ishida Y, Akamatsu E, Ohmori Y, Sudoh M, Uto H, et al. Proanthocyanidin from blueberry leaves suppresses expression of subgenomic hepatitis C virus RNA. The Journal of Biological Chemistry. 2009;**284**:21165-21176
- [54] Ali BH, Al Wabel N, Blunden G. Phytochemical, pharmacological and toxicological aspects of *Hibiscus sabdariffa* L.: A review. Phytotherapy Research. 2005;**19**(5):369-375
- [55] Morton J. Roselle. In: Fruits of Warm Climates. Miami, FL: Creative Resource Systems Inc.; 1987. pp. 281-286
- [56] Tsai P-J, McIntosh J, Pearce P, Camden B, Jordan BR. Anthocyanin and antioxidant capacity in Roselle (*Hibiscus sabdariffa* L.) extract. Food Research International. 2002;**35**(4):351-356
- [57] Lin HH, Huang HP, Huang CC, Chen JH, Wang CJ. Hibiscus polyphenol-rich extract induces apoptosis in human gastric carcinoma cells via p53 phosphorylation and p38MAPK/FasL cascade pathway. Molecular Carcinogenesis. 2005;**43**(2):86-99
- [58] McKay DL, Chen CY, Saltzman E, Blumberg JB. *Hibiscus sabdariffa* L. tea (tisane) lowers blood pressure in prehypertensive and mildly hypertensive adults. Journal of Nutrition. 2010;**140**(2):298-303
- [59] Yang YS, Wang CJ, Huang CN, Chen ML, Chen MJ, Peng CH. Polyphenols of *Hibiscus sabdariffa* improved diabetic nephropathy via attenuating renal epithelial mesenchymal transition. Journal of Agriculture and Food Chemistry. 2013;**61**(31):7545-7551
- [60] Liu KS, Tsao SM, Yin MC. In vitro antibacterial activity of roselle calyx and protocatechuic acid. Phytotherapy Research. 2005;**19**(11):942-945
- [61] Borges A, Ferreira C, Saavedra MJ, Simoes M. Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria. Microbial Drug Resistance. 2013;**19**(4):256-265
- [62] Saini P, Gayen P, Nayak A, Kumar D, Mukherjee N, Pal BC, et al. Effect of ferulic acid from *Hibiscus mutabilis* on filarial parasite *Setaria cervi*: Molecular and biochemical approaches. Parasitology International. 2012;**61**(4):520-531
- [63] Kassem AF, Shaheen MNF, Batran RZ, Abbas EMH, Elseginy SA, Elmahdy EM. New 4-phenylcoumarin derivatives as potent 3C protease inhibitors: Design, synthesis, anti-HAV effect and molecular modeling. European Journal of Medicinal Chemistry. 2019;**168**:447-460
- [64] Batran RZ, Kassem AF, Abbas EMH, Elseginy SA, Mounier MM. Design, synthesis and molecular modeling of new 4-phenylcoumarin derivatives as tubulin polymerization inhibitors targeting MCF-7 breast cancer cells. Bioorganic and Medicinal Chemistry. 2018;**26**:3474-3490

- [65] Garro HA, Pungitore CR. Coumarins as potential inhibitors of DNA polymerases and reverse transcriptases. Searching new antiretroviral and antitumoral drugs. *Current Drug Discovery Technologies*. 2015;**12**:66-79
- [66] Bedoya LM, Beltran M, Sancho R, Olmedo DA, Sanchez-Palomino S, del Olmo E, et al. 4-Phenylcoumarins as HIV transcription inhibitors. *Bioorganic & Medicinal Chemistry Letters*. 2005;**15**:4447-4450
- [67] Marquez N, Sancho R, Bedoya LM, Alcamí J, Lopez-Perez JL, Feliciano AS, et al. Mesuol, a natural occurring 4-phenylcoumarin, inhibits HIV-1 replication by targeting the NF-kappa B pathway. *Antiviral Research*. 2005;**66**:137-145
- [68] Gill TA, Singer DS, Thompson JW. Purification and analysis of protamine. *Process Biochemistry*. 2006;**41**:1875-1882
- [69] Islam NMD, Oda H, Motohiro T. Changes in the cell morphology and the release of soluble constituents from washed cells of *Bacillus subtilis* by the action of protamine. *Nippon Suisan Gakkaishi*. 1987;**53**:297-303
- [70] Behçet A. The source-synthesis-history and use of atropine. *The Journal of Academic Emergency Medicine*. 2014;**13**:2-3
- [71] Win NN, Kanda T, Nakamoto S, Moriyama M, Jiang X, Suganami A, et al. Inhibitory effect of Japanese rice-koji miso extracts on hepatitis A virus replication in association with the elevation of glucose-regulated protein 78 expression. *International Journal of Medical Sciences*. 2018;**15**(11):1153-1159
- [72] Jiang X, Ren Q, Chen SP, et al. UVC mutagenicity is suppressed in Japanese miso-treated human RSa cells, possibly via GRP78 expression. *Bioscience, Biotechnology, and Biochemistry*. 2011;**75**:1685-1691
- [73] Jiang X, Kanda T, Haga Y, et al. Glucose-regulated protein 78 is an antiviral against hepatitis A virus replication. *Experimental and Therapeutic Medicine*. 2017;**13**:3305-3308
- [74] Park SY, Ha S-D. Inactivation of murine norovirus-1 and hepatitis A virus in the Korean traditional preserved raw crab product Ganjanggejang by soy sauce during storage. *Food Control*. 2015;**51**:293-299
- [75] Choi NS, Chung SJ, Choi JY, Kim HW, Cho JJ. Physico-chemical and sensory properties of commercial Korean Traditional Soy sauce of mass produced vs. small scale farm produced in the Gyeonggi area. *The Korean Journal of Food and Nutrition*. 2013;**26**:553-564
- [76] Masuda S, Hara-Kudo Y, Kumagai S. Reduction of *Escherichia coli* O157:H7 populations in soy sauce, a fermented seasoning. *Journal of Food Protection*. 1998;**61**:657-661
- [77] Kim JS, Moon GS, Lee YS. Chromaticity and brown pigment patterns of soy sauce and UHYUKJANG, Korean traditional fermented soy sauce. *Korean Journal of Food and Cookery Science*. 1996;**23**:642-649
- [78] Mao S, Wang M, Ou X, Sun D, Cheng A, Zhu D, et al. Virologic and immunologic characteristics in mature ducks with acute duck hepatitis A virus 1 infection. *Frontiers in Immunology*. 2017;**8**:1574
- [79] Song M, Chen Y, Du H, Zhang S, Wang Y, Zeng L, et al. Raw *Rehmannia radix* polysaccharide can effectively release peroxidative injury induced by duck hepatitis A virus. *African Journal of Traditional, Complementary, and Alternative Medicines*. 2017;**14**(4):8-21

[80] Ming K, Chen Y, Yao F, Shi J, Yang J, Du H, et al. Phosphorylated *Codonopsis pilosula* polysaccharide could inhibit the virulence of duck hepatitis A virus compared with *Codonopsis pilosula* polysaccharide. International Journal of Biological Macromolecules. 2017;**94**:28-35

[81] Chen Y, Yang Y, Wang F, Yang X, Yao F, Ming K, et al. Antiviral effect of baicalin phospholipid complex against duck hepatitis A virus type 1. Poultry Science. 2018;**97**(8):2722-2732

[82] Chen Y, Zeng L, Lu Y, Yang Y, Xu M, Wang Y, et al. Treatment effect of a flavonoid prescription on duck virus hepatitis by its hepatoprotective and antioxidative ability. Pharmaceutical Biology. 2017;**55**(1):198-205