Chapter

Antiviral Natural Products against Hepatitis-A Virus

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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 antiviral 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 novovirus, 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 it's 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 (IC_{50} = 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 (IC_{50} = 8.5 μg/ml, TI =88; IC_{50} = 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 (Ki = 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 trituberculantus*) 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 *Hibiscus sabdariffa* 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.

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