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Comparison of the virucidal efficacy of essential oils (cinnamon, clove, and thyme) against hepatitis A virus in suspension and on food-contact surfaces

Md Iqbal Hossain, Zhaoqi Wang, Daseul Yeo, Soontag Jung, Hyojin Kwon, Yuan Zhang, Danbi Yoon, Seongwon Hwang, Changsun Choi *

Department of Food and Nutrition, College of Biotechnology and Natural Resources, Chung-Ang University, Anseong, Gyeonggi-do, 17546, Republic of Korea

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ABSTRACT

Essential oils (EOs) have been used for centuries as flavor enhancers in foods, and owing to their antimicrobial properties, they have potential as natural food preservatives. However, their effect on food-borne viruses is unknown. Therefore, in this study, the virucidal effects of three EOs (cinnamon, clove, and thyme) on the infectivity of the hepatitis A virus (HAV) were investigated. Different concentrations of each EO (0.05, 0.1, 0.5, and 1%) were mixed with viral suspensions in accordance with ASTM E1052–11:2011 and incubated for 1 h at room temperature. The EOs exhibited a concentration-dependent effect in the suspension tests, and HAV titers decreased by approximately 1.60 log PFU/mL when treated with EOs at the highest concentration of 1%. The antiviral effect of EOs treated at 1% for 1 h was also evidenced in surface disinfection tests according to the OECD:2013, as approximately 2 log PFU/mL reduction on hard food-contact surfaces (stainless steel and polypropylene) and approximately 2 and 1.4 log PFU/mL reduction on low-density polyethylene and kraft (soft food-contact surfaces), respectively. Moreover, RT-qPCR results revealed that HAV genome copies were negligibly reduced until treated with a high concentration (1%) in suspension and carrier tests. Overall, our findings highlighted the potential of cinnamon, clove, and thyme EOs as natural disinfectants capable of limiting HAV (cross-) contamination conveyed by food-contact surfaces. These findings advance our knowledge of EOs as antimicrobials and their potential in the food sector as alternative natural components to reduce viral contamination and improve food safety.

1. Introduction

According to a recent review of the literature on food microbiology, the impact of viruses on food is the least studied topic (Bermudez-Aguirre, 2020). This is because laboratories focusing on food microbiology often lack the necessary equipment and security measures (Jayasena et al., 2015). Owing to their inability to proliferate or alter the organoleptic properties of food in the absence of a living host cell, viruses seldom cause foodborne illnesses. However, regulatory authorities and the global food sector are concerned about viral foodborne illnesses (Bosch et al., 2018). According to the World Health Organization (WHO), norovirus (NoV) and hepatitis A virus (HAV) are the most common viruses that cause foodborne illnesses (Yadav et al., 2010). The fecal–oral route is the most common means of transmission for these viruses, followed by casual human contact, and contaminated water and food. Food can be contaminated with viruses that cause human illnesses (D'Souza and Joshi, 2016). Food contamination can occur at any stage of the supply chain, from the farm to the table (Acheson and Fiore, 2004). The WHO has reported over 600 million cases of foodborne illnesses and 420,000 related deaths worldwide (WHO, 2022). Non-enveloped foodborne viruses such as HAV are considered a global public health risk, and efforts are underway to prevent and control their transmission. Over the course of a few years, aggressive strains of these rapidly spreading viruses will evolve because of mutations. Viral illnesses continue to represent the foremost global risk to human health. To date, only a few medications have proven effective against various viruses, necessitating the exploration of new antiviral lead compounds. Thus, new and efficient methods are required to halt the proliferation and transmission of infection.

Essential oils (EOs) are oily liquids with distinct aromas extracted from different plant parts through various processes. Important antiseptic, antioxidant, and antimicrobial properties have been attributed to EOs (Chouhan et al., 2017; Ma and Yao, 2020). Numerous EOs have been shown to be effective against many different types of viruses (Allahverdiyev et al., 2004). In the European Union (EU), EOs and their

* Corresponding author.
E-mail address: cchoi@cau.ac.kr (C. Choi).

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Glossary

WHO	World Health Organization
NoV	norovirus
HAV	Hepatitis A virus
EOs	Essential oils
EU	European Union
OECD	Organisation for Economic Co-operation and Development
FRhK-4	Fetal rhesus monkey kidney
ATCC	American Type Culture Collection
DMEM	Dulbecco's Modified Eagle's Medium
$NaHCO_3$	Sodium bicarbonate
FBS	Fetal bovine serum
AA	Antibiotic/Antimycotic
CO_2	Carbon dioxide
PFU	Plaque forming units
DMSO	Dimethylsulfoxide
BSA	Bovine serum albumin
PBS	Phosphate buffered saline
SS	Stainless steel
PP	Polypropylene
LDPE	Low-density polyethylene
RT-qPCR	Reverse transcriptase quantitative polymerase chain reaction
GC	Genome copy
Cq	Quantification cycle
FDA	Food and Drug Administration

volatile components are most commonly used in pharmaceuticals, food (as flavorings and antioxidants), and perfumes (fragrances and aftershaves) (Burt, 2004). Numerous studies have demonstrated the effectiveness of EOs in combating harmful microbes (Ozogul et al., 2015; Piątkowska and Rusiecka-Ziołkowska, 2016). However, limited data are existing on the efficacy of EOs alongside foodborne viruses, and few EOs have been examined for their application in the food sector (Elizaquível et al., 2013; Kim et al., 2017; Kovač et al., 2012; Sánchez and Aznar, 2015) mainly against HAV (Sánchez and Aznar, 2015).

Additionally, food undergoes numerous processing steps between harvest and consumption, which increases the possibility of it being cross-contaminated by unclean surfaces or by people who have handled it. When handling prepared food, contaminated surfaces must be cleaned properly to lower the possibility of foodborne illnesses. Therefore, maintaining a clean environment while processing food is essential. The use of EOs as "natural" additives can be a viable option for sanitizing food products. EOs can be exploited in food sector due to their antimicrobial properties (Chouhan et al., 2017; Ma and Yao, 2020). This can help avoid future HAV-related illnesses by minimizing HAV contamination in food-processing facilities. However, methods for cleaning potentially infected surfaces are needed to achieve this success. Using the carrier testing method adapted from the procedure specified by Organisation for Economic Co-operation and Development (OECD), we evaluated the potential use and effectiveness of three EOs, derived from cinnamon leaves (*Cinnamomum zeylanicum* Blume), cloves (*Eugenia* spp.), and thyme (*Thymus vulgaris* or *Thymus zygis*), to reduce or eliminate viral contamination during food processing by reducing viral loads of HAV on food-contact surfaces.

2. Materials and methods

2.1. Viruses and cells

The HAV strain HM-175 (ATCC VR-1402) and fetal rhesus monkey kidney (FRhK-4, ATCC CRL-1688) cells were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). In an incubator (37 °C with 5% CO_2), FRhK-4 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Sigma-Aldrich, USA) with sodium bicarbonate ($NaHCO_3$), 10% fetal bovine serum (FBS; Gibco, NY, USA), and 1% antibiotics-antimycotics (AA; Gibco) in culture flasks (SPL, Republic of Korea). The cells were sub-cultured every three days. Plaque assays were used to prepare HAV suspensions at a titer of 1.8×10^7 log Plaque forming units (PFU)/mL after culturing on 90% confluent FRhK-4 cells for 6–7 days in an incubator (37 °C with 5% CO_2) (Song et al., 2022).

2.2. Virus titration

Quantitative HAV titers were measured using a plaque assay in 12-well plates (SPL, Republic of Korea) as previously described, with minor modifications (Song et al., 2022). HAV suspensions were diluted 10-fold in serum-free DMEM, and diluted HAV suspension (500 μL) was added to each well (90% confluence of FRhk-4 cells). Subsequently, the protocol described by Song et al. (2022) was followed for conducting the plaque assay. HAV titers were expressed as log PFU/mL after plaque evaluation.

2.3. Preparation of the inoculum, essential oils (EOs), and food-contact surfaces

The inoculum (500 μL) was prepared using 5% of soil-load stock solution each, i.e., 100 μL mucin, 35 μL yeast extract, 25 μL bovine serum albumin (BSA), and 340 μL of viral suspension (HAV titer 1.8×10^7 log PFU/mL), according to the OECD guideline (OECD, 2013; Min et al., 2023).

Each EO (cinnamon leaf, clove, and thyme from Sigma-Aldrich) was emulsified as described by Hossain et al. (2022). The EOs were solubilized in 0.5% (v/v) Tween-80 (Sigma-Aldrich) as a surfactant and 10% (v/v) dimethylsulfoxide (DMSO; Sigma-Aldrich). Stock solutions of 10% (v/v) were prepared for each EO. After 1 h of stirring, an emulsion was obtained from the mixture. The solubility of the EO was improved by the addition of DMSO, and the emulsion was made more stable by the addition of Tween-80.

Hard food-contact surfaces: stainless steel (SS) and polypropylene (PP) discs (1 mm thickness and 1 cm width) as carriers bought from Leehyo Bioscience Co., Ltd. (Seongnam, Republic of Korea). Soft food-contact surfaces: the kraft paper and low-density polyethylene (LDPE) surfaces were purchased from an online market and turned into carriers with a width of 8 mm using a punch (8 mm Biopsy punch, Miltex, USA). All surfaces were sterilized in an autoclave, except for LDPE. LDPE was degreased with acetone, sterilized by soaking in 70% EtOH and dried in a clean bench with laminar flow (HB-402; Han Baek Scientific Co., Bucheon, Republic of Korea) (Jung et al., 2023).

2.4. Cytotoxicity of EOs against FRhK-4 cells

The cell counting kit 8 (CCK-8) (Sigma-Aldrich) was used to determine EOs cytotoxicity. FRhK-4 cells were seeded in 96-well plates at a density of 10,000 cells/well and incubated at 37 °C with 5% CO_2 for 48 h in an incubator; the cells were then treated with 10 μL of each EO at a concentration of 0.01–1%. The 96-well plate was incubated in a CO_2 incubator at 37 °C for 24 h, and 10 μL of CCK-8 solution was then added to each well of the plate. After 4 h of incubation, the absorbance was measured at 450 nm using an Epoch spectrophotometer (BioTek,

Winooski, VT, USA). The cell viability percentage was calculated as follows:

Cell viability (%) = $(OD_{test} - OD_{blank})/(OD_{control} - OD_{blank}) \times 100$ (Seo et al., 2017).

2.5. Suspension test

A suspension test adapted from the ASTM E1052-11 standard method with slight modifications was used to evaluate the activation impact of the three EOs (ASTM E1052-11, 2011). The different tested concentrations of EOs (900 μL) or control (900 μL; serum-free DMEM) were mixed with inoculum (100 μL) from the earlier prepared (500 μL). After 1 h of exposure, the suspension was vortexed for 10 s and transferred (100 μL) to the neutralizer (900 μL; serum-free DMEM) for neutralizing the test substances. The suspension was then vortexed to recover the HAV, and plaque assays were performed on the FRhK-4 host cells to determine the viral titers. The formula described by Song et al. (2022) was used to determine the log reduction in HAV titers. The plaque assay was used to determine the antiviral effectiveness of the EOs.

2.6. Quantitative carrier test

A quantitative carrier test adapted from the OECD procedure with minor adjustments was conducted to evaluate the activation impact of the three EOs (OECD, 2013). Briefly, 10 μL of the inoculum from the previously prepared (500 μL) was dropped onto each carrier (surface) and dried (1 h) in a clean bench with laminar flow (HB-402; Han Baek Scientific Co., Bucheon, Republic of Korea). The carrier was treated with 50 μL of different EO concentrations for 1 h. The carrier was directly shifted to 950 mL of serum-free DMEM to neutralize the EO; the samples were then vortexed (1 min) to recover HAV. Subsequently, plaque assays were performed on FRhK-4 host cells to determine the viral titers. The formula described by Song et al. (2022) was used to determine the log reduction in HAV titers.

2.7. RNA extraction and RT-qPCR

The RNeasy Mini Kit (QIAGEN, Hilden, Germany) was used for RNA extraction according to the manufacturer's instructions. Viral RNA was extracted from half of the final solution (total volume, approximately 0.5 mL). RNA was quantified by RT-qPCR on the same day using a CFX96 Touch Real-Time PCR Detection System (C1000 Touch Thermal Cycler Dice, USA). A one-step RT-qPCR kit (QuantiTect Probe RT-PCR Kit, QIAGEN) was used to perform the one-step RT-qPCR. The RNA sample was amplified in 25 μL reaction mixture, containing 12.5 μL of Master Mix (2X) (QuantiTect Probe RT-Master Mix, QIAGEN), 0.25 μL of RT Mix (QuantiTect RT Mix, QIAGEN), 1.25 μL primer-probe mixture (20X) (IDT, Integrated DNA Technologies), and RNase-free water added up to 25 μL. The primer and probe sequences were as follows (5′–3′): forward primer (GGTAGGCTACGGGTGAAAC), reverse primer (AACAACTCACCAATATCCGC), probe (FAM-CTTAGGCTAA-TACTTCTATGAAGAGATGC-TAMRA). The settings were as follows: 15 min reverse transcription of RNA at 50 °C, followed by a 15 min denaturation step at 95 °C, and finally 45 cycles of 10 s at 95 °C, 20 s at 55 °C, and 1 min at 65 °C. The RT-qPCR and thermal cycle conditions were those that Wang et al. (2023) recommended. Quantification was performed using standard quantitative genomic viral RNA from ATCC (HAV, VR-3257SD); the slope and intercept of HAV were evaluated using the formula: $Y = -3.507 \times \log(x) + 41.86$.

The quantification cycle (Cq) values of each reaction were converted to genome copies (GC), as previously described (Wang et al., 2022, 2023).

2.8. Statistical analysis

All tests were executed thrice, and the mean reduction (log PFU/mL, log GC/sample) was used to compile the data. Means and standard deviations are used to display data. Software from GraphPad Software, Inc. was used to create the graphs in this study. Statistical significance was evaluated using one-way ANOVA, followed by Duncan's Post-hoc test using SPSS statistics v26.0 (IBM, NY, USA). A P value < 0.05 was considered statistically significant.

3. Results

3.1. Determination of cytotoxicity on cell monolayers

The strength and diversity of cellular morphological fluctuations were found to be dose-dependent (Lanave et al., 2017). After exposure to various doses of each EO (0.01–1%) for 24 h, morphological examination and the CCK-8 assay results revealed that the viability of FRhK-4 cells was substantially reduced. However, cell viability did not decrease significantly at low EO concentrations. In FRhK-4 cells, EOs at doses >0.1% were cytotoxic.

3.2. Effect of EOs on HAV infectivity in suspension

Plaque reduction assay and RT-qPCR analysis were used to evaluate whether EOs could prevent HAV attachment to host cells or block HAV entry into host cells. Plaque reduction assay revealed that increasing concentrations of EOs (0, 0.05, 0.1, 0.5, and 1%, v/v) dose-dependently reduced the viral titers (Fig. 1); notably, HAV titers decreased by 1.61, 1.55, and 1.65 log PFU/mL when treated with cinnamon, clove, and thyme EO at a concentration of 1%, respectively, whereas HAV titers decreased only by 1.28, 1.33, and 1.32 log PFU/mL at 0.5% concentration with the same three EOs. However, the suspension test indicated a reduction of <1 log PFU/mL in the HAV titer at 0.05% and 0.1% concentrations of all three EOs. High concentrations (0.5% and 1%) of EOs were more effective than low concentrations in reducing HAV infection (Fig. 1).

3.3. Effect of EOs on HAV infectivity on hard food-contact surfaces

Fig. 2 displays the decline in the infectious titers of HAV inoculated on SS and PP with and without adding EOs as surface sanitizers. The

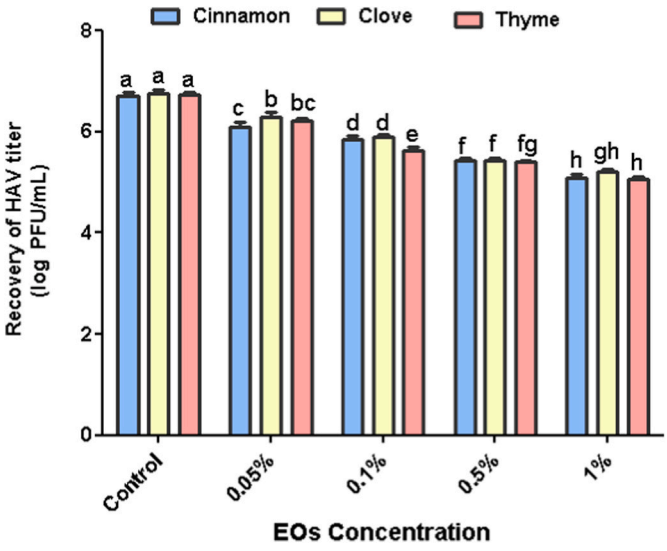

Fig. 1. Effect of EOs (cinnamon, clove, and thyme) on HAV infectivity in suspension.

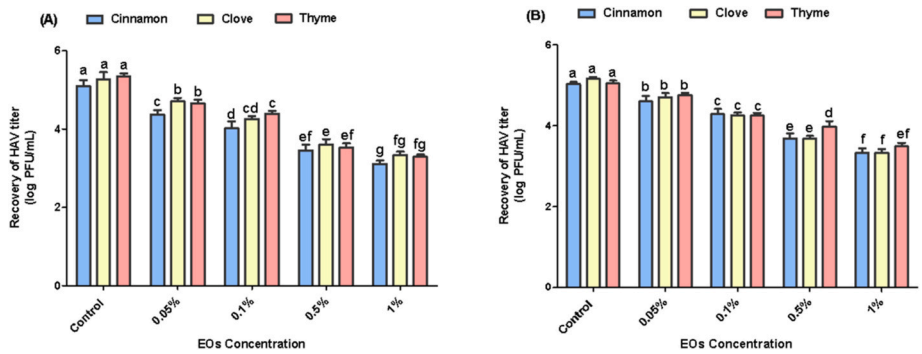

Fig. 2. Effect of EOs (cinnamon, clove, and thyme) on HAV infectivity on hard food-contact surfaces: (A) stainless steel (SS) (B) polypropylene (PP).

viral titer of HAV was lower in samples treated with all three EOs for 1 h at room temperature compared with that in the control sample. These results suggest that the anti-HAV activity of the EOs is dose-dependent (Fig. 2). Notably, on SS, the HAV titer was reduced by 1.98, 1.94, and 2.06 log PFU/mL after treatment with cinnamon, clove, and thyme EOs at 1.0%, by 1.64, 1.67, and 1.83 log PFU/mL after treatment with the same EOs at 0.5%, respectively. On PP, the HAV titer was reduced by 1.98, 1.94, and 2.06 log PFU/mL after treatment with cinnamon, clove, and thyme EOs at 1.0%, by 1.64, 1.67, and 1.83 log PFU/mL after treatment with the same EOs at 0.5%, respectively. Samples treated with 0.5 and 1% EOs exhibited less HAV on SS and PP than those in the control group. However, a reduction of <1 log PFU/mL in the HAV titer decrease between the EO-treated and control (non-treated) samples were seen when the treatments were carried out at low concentrations (0.05% and 0.1%) (Fig. 2).

3.4. Effect of EOs on HAV infectivity on soft food-contact surfaces

The effect of EO treatment on soft food-contact surfaces contaminated with HAV is displayed in Fig. 3. HAV titer were decreased compared with those in the control during EOs treatment. Upon treatment with cinnamon EO at 0.05, 0.1, 0.5, and 1%, the HAV titer was reduced by 0.46, 0.72, 1.07, and 2.16 log PFU/mL on LDPE, and by 0.40, 0.76, 1.09, and 1.41 log PFU/mL on kraft, respectively. Upon treatment with clove EO at 0.05, 0.1, 0.5, and 1%, the HAV titer was reduced by 0.31, 0.64, 1.37, and 1.91 log PFU/mL on LDPE, and by 0.31, 0.51, 0.73, and 1.11 log PFU/mL on kraft, respectively. Upon treatment with thyme EO at 0.05, 0.1, 0.5, and 1%, the HAV titer was reduced by 0.98, 1.13, 1.75, and 2.15 log PFU/mL on LDPE, and by 0.30, 0.49, 1.04, and 1.17 log PFU/mL on kraft, respectively. However, high concentrations (0.5% and 1%) of EOs were more effective than low concentrations (0.05% and 0.1%) in reducing HAV infection (Fig. 3).

3.5. Effect of EOs on the genomic copy changes of HAV in the suspension and carrier (food-contact surface) tests

Reduced viral infectivity following EO treatment in the suspension test was further validated using RT-qPCR. The viral loads of HAV in the suspension test were quantified as approximately 6.2 log GC/sample. The RT-qPCR results revealed that the HAV GC/sample were reduced by a maximum of 0.2–0.3 log values between the control and high EO concentration (1%) during treatment (Fig. 4).

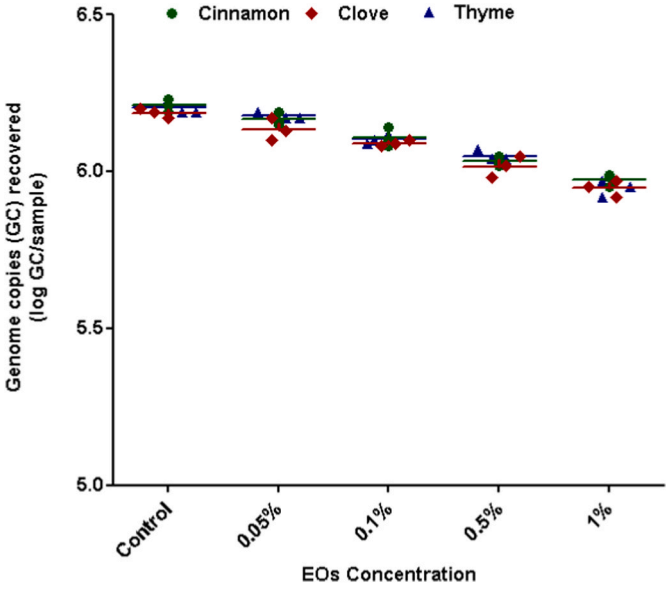

Fig. 4. Effect of EOs (cinnamon, clove, and thyme) on the genomic copies changes of HAV in suspension detected via RT-qPCR.

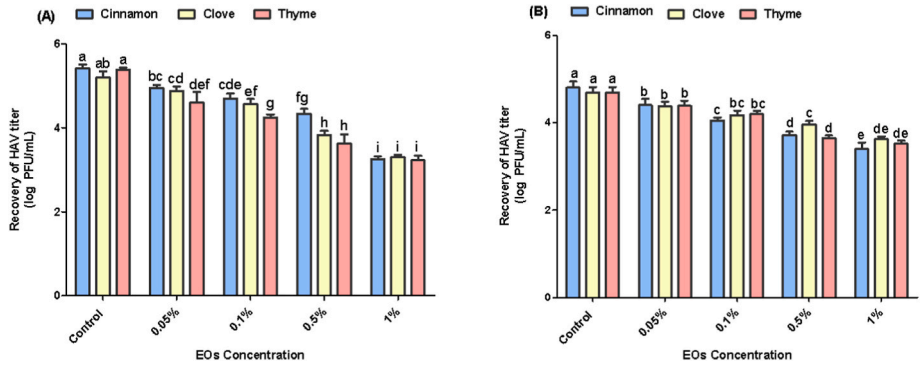

Fig. 3. Effect of EOs (cinnamon, clove, and thyme) on the infectivity of HAV on soft food-contact surfaces: (A) low-density polyethylene (LDPE) (B) kraft.

Fig. 5 displays the results of an RT-qPCR analysis for the viral recovery efficiency after spiking HAV on food contact surfaces. Approximately 5.5 and 5.3 log GC/sample of HAV were detected initially on SS and kraft paper, respectively. However, the amount of HAV GC/sample were reduced by a maximum of 0.3–0.4 log values between the control and high EO concentration (1%) on SS and kraft paper during treatment (Fig. 5).

4. Discussion

EOs have long been utilized in the perfume and cosmetic sectors, and as food and beverage preservatives and taste enhancers (Hyldgaard et al., 2012). Further, owing to their virucidal properties, these chemicals are used in food and healthcare divisions to thwart the spread of viruses (Pellegrini et al., 2023). *In vitro* antiviral activity tests often involve reduced cytopathogenic effects, viral plaques, and viral yield (Ahmed et al., 2014). Antivirals act by impeding virus growth in a concentration-dependent way, and testing cytotoxicity of EOs is essential to ensure that they do not harm the host cells at the amount used in the assay. Studies on EO toxicity are necessary because EOs may be poisonous when used in excess and negatively affect human cells. For instance, several studies have reported cytotoxic effects of EOs in eukaryotic cells (Ahmed et al., 2011; Nathalie et al., 2006).

A key risk factor for food contamination is the ability of foodborne viruses to survive on surfaces that come in contact with food. The problem with the assay, involving a quantitative suspension test in an experimental tube, is that the virus elements are disseminated in an abundance of antiviral agents, causing the virus to deactivate more quickly owing to the extensive interaction between antiviral agents and viral particles. Overall, suspension tests do not reflect real-world circumstances accurately. Further, viruses attach to proteins abundant in physiological fluids, which may shield them from antiviral agents. Therefore, testing their effectiveness in settings comparable to actual field circumstances is necessary to ensure that surface disinfectants can kill microbes. The effectiveness of disinfectants in killing bacteria has been determined using this practical and realistic approach (Rabenau et al., 2014). Hence, a virucidal carrier assessment that mimics real-world circumstances is needed to validate this hypothesis and gain further insights. We conducted a carrier (food-contact surface) test following a modified OECD procedure and compared the results with those obtained from suspension tests to determine which EOs were the most effective against HAV.

In HAV suspensions in tissue culture medium, a minimal decrease (<1 log) in virus infectivity was observed when treated with oregano and zataria EOs (Sánchez and Aznar, 2015). Carvacrol treatment reduced less than 1 log HAV infectivity at the highest concentration examined (1%) after 2 h at 37 °C (Sánchez et al., 2015). HAV titers were reduced by 1 log after 2 h at 37 °C and by 3.4 log after overnight incubation when 1% cinnamaldehyde was used, and by 2.7 log after overnight incubation when 0.5% cinnamaldehyde was used (Fabra et al., 2016). Several natural additives have been investigated to lower HAV infectivity but have not been successful (Sánchez et al., 2015; Sánchez and Aznar, 2015; Lee et al., 2013). In addition, grape seed extract (GSE), blueberry juice, blueberry proanthocyanidins, cinnamaldehyde, and green tea extract (GTE) have demonstrated promising results in various studies (Randazzo et al., 2017; Joshi et al., 2016; Fabra et al., 2016). Our understanding of the effectiveness of EOs as an alternate solution to lessen the likelihood of HAV contamination of food-contact surfaces remains limited or unknown. This may be partially attributed to the growing customer demand for efficient, affordable, safe, and natural products, as well as increased knowledge regarding the frequency of enteric viruses in food products. Recently, the use of EOs has attracted growing interest in the preservation of fresh fruits because of their comparatively safe nature (Vergis et al., 2015). The HAV titer was reportedly reduced on the surface of treated berries after exposure to 0.5% lemon (2.84 log), 0.1% grapefruit (2.89 log), and rosemary cineole (2.94 log) (Battistini et al., 2019). These substances are well-established EOs for food preservation (Martínez et al., 2018; Lee et al., 2019). Here, we evaluated the antiviral effect of cinnamon, clove, and thyme EOs at different concentrations ranging from 0.05 to 1.0% against HAV on soft and hard food-contact surfaces, as well as in suspension tests. Our findings suggested that cinnamon, clove, and thyme EOs substantially decreased HAV titers. As the concentration of EOs increased, HAV titers decreased in a dose-dependent manner, as shown in this study. Although viral RNA persisted in our study with almost no changes, viral viability was lost during the treatment. As naked residual viral RNA lacks infectious potential and rapidly dissolves in the environment, it does not pose a threat to the safety of food, dinnerware, or human health (McCall et al., 2022).

Effective virucidal agents kill viruses through various mechanisms, including cell structure modification, protein aggregation, and denaturation (Boyce, 2018). Although molecular approaches have been utilized to study the virucidal effects and inactivation mechanisms, loss of infectivity remains the standard method for gauging the success of deactivation processes (Baert et al., 2008). Mechanical assets of the viral capsid, including flexibility and ability to tolerate stress, such as DNA wrapping and cell entry, and extracellular variables significantly impact viability (Carrasco et al., 2011; Greber, 2016). The action mechanisms of EOs are more diverse than those of synthetic antiviral constituents, which function in a manner specific to a specific virus type (Lai et al., 2012). The activity of EOs can impede the capacity of virus to infiltrate host cells at the extracellular level by disrupting the structure of the viral envelope or blocking viral proteins. Additionally, EOs potentially exert antiviral effects against intracellular viruses (Ma and Yao, 2020). To date, insufficient evidence regarding the mechanism underlying the antiviral activity of EOs has hampered their widespread use. Typically, researchers can only determine the stage of viral infection at which the EOs act. To harness the potential of EOs against viral infections,

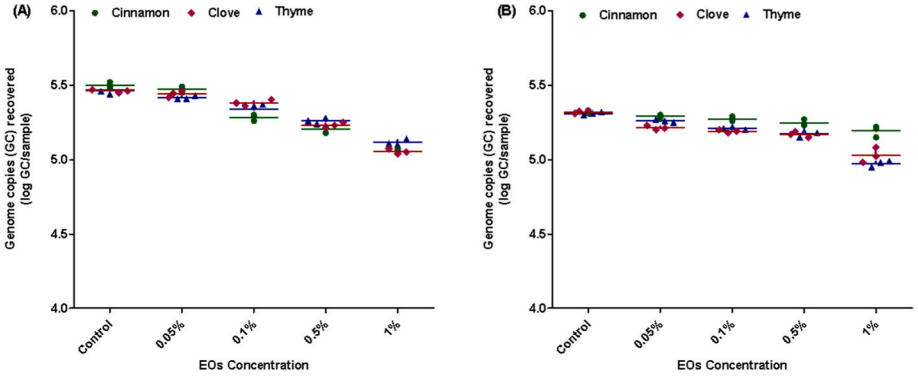

Fig. 5. Effect of EOs (cinnamon, clove, and thyme) on the genomic copy changes of HAV on (A) SS and (B) Kraft paper detected via RT-qPCR.

understanding these pathways is crucial (Ma and Yao, 2020). Therefore, in the future, understanding the underlying mechanisms by which EOs inhibit viral infections is the most crucial.

Overall, our findings add to the current understanding of the antiviral action of EOs and emphasize their potential for use in reducing HAV infections through suspensions and food-contact surfaces. However, our study also has the following limitations: owing to variations in experimental settings (such as temperature, virus particle number, and EO/virus contact period), comparing the outcomes of this study with those of previous investigations is challenging. As EOs rapidly evaporate in the presence of heat, light, oxygen, and humidity, their conservation is another constraining issue. The inherent characteristics of virus cultivation are also subject to a few constraints. Further, conducting this research on a smaller scale in the laboratory and performing more in-depth investigations with a larger number of experimental samples are required to comprehensively evaluate whether EO-food reactions may be successfully utilized in the food sector without creating any harmful byproducts. Future research is also necessary to determine whether EOs inactivate other foodborne and waterborne viruses, such as norovirus, rotavirus, and adenovirus, and to clarify the specifics of their virus-inactivation mechanisms. Notably, consumers prefer EOs as sanitizing agents over synthetic chemicals because they are approved as additives by Food and Drug Administration (FDA), preserve the nutritious qualities of food, have fewer adverse effects on the environment, and are less harmful (not all natural EOs) to humans. However, these techniques alone are insufficient to inactivate or decontaminate foods infected with high viral loads. Therefore, the use of EOs in conjunction with other treatments for food and food-contact surfaces must be considered. Additional research is required to determine the lowest amount of EO required to retain antiviral action without altering the organoleptic properties of food products. To guarantee the safety of food products without disrupting production timing according to current industrial practices, the minimal time required by EOs to diminish the highest HAV titers must also be estimated. Finally, additional research is required to examine the mechanisms by which EOs affect the organoleptic qualities of a product throughout its storage and distribution.

5. Conclusion

We compared the virucidal efficiencies of three EOs (cinnamon, clove, and thyme) against HAV using suspension and carrier tests. The effective dosages of the EOs evaluated here provide a valuable starting point for considering these substances as potentially active surface disinfectants. Our results also indicate that suspension tests were less effective than carrier tests in estimating the actual surface disinfectant level. Our findings can also be used to supply the food industry with accurate information and help reduce food contamination. Further, they demonstrate that the carrier testing method can help assess the antiviral effectiveness of EOs and ensure food safety. Additional research is required on the broad screening of novel antiviral agents, comprehensive elucidation of their action mechanisms, as well as their possible application in food and food processing. Overall, the data obtained in this study are invaluable for ensuring the safety of food-contact surfaces for which HAV contamination is a concern.

CRediT authorship contribution statement

Md Iqbal Hossain: Data curation, Formal analysis, Investigation, Visualization, Writing – original draft, Funding acquisition. **Zhaoqi Wang:** Investigation. **Daseul Yeo:** Investigation. **Soontag Jung:** Investigation. **Yuan Zhang:** Validation. **Danbi Yoon:** Validation. **Seongwon Hwang:** Validation. **Changsun Choi:** Conceptualization, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that may have influenced the work reported in this study.

Data availability

The authors do not have permission to share data.

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