

Natural Virucidal Compounds in Foods

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1. INTRODUCTION

Numerous plants have been shown to possess significant antibacterial, antifungal, antiviral, insecticidal, antioxidant, and anti-cancer properties (Didry et al. 1994; Friedman et al. 2002; Hammer et al. 2002; Knowles et al. 2005; Kordali et al. 2005; Peñalver et al. 2005; Carson et al. 2006; Pinto et al. 2006; Callaway et al. 2008; Ravishankar et al. 2008; Ravishankar et al. 2009; Reichling et al. 2009; Ravishankar et al. 2010). Natural antimicrobial compounds are produced in various parts of the plants e.g., flowers, buds, fruits, seeds, herbs, roots, leaves, bark, wood, and stem, coinciding with the various assaults that the plant might encounter in the environment (Burt 2004). The fragrance of plants is carried in the quinta essential, or essential oil fraction (Cowan 1999). These volatile oils are aromatic, viscous liquids (Burt 2004) that are complex mixtures of lipophilic and volatile secondary metabolites such as monoterpenes, sesquiterpenes, and/or phenylpropanoids. They are primarily responsible for a plant's fragrant and biological properties (Reichling et al. 2009).

Essential oils and other components may be separated from plants through processes such as extraction (liquid-liquid extraction, solid-phase extraction, supercritical fluid extraction, pressurized liquid extraction, microwave-assisted extraction, and ultrasound-assisted extraction), distillation, and cold pressing (Burt 2004; Rasooli 2007; Garcia-Salas et al. 2010). Of these, steam distillation is the most commonly employed commercial method for extraction of essential oils (van de Braak and Leijten 1999).

Essential oils are typically mixtures of many compounds and may have as many as 60 individual components (Senatore 1996; Russo et al. 1998). The composition can change depending on the geographical location, the soil, and even the season, leading to the biosynthesis of different metabolites. The active ingredient is often the dominant component, at times accounting for greater than 50 % of its chemical composition (Burt 2004). For instance, the carvacrol content of oregano oil may be as high as 85 % and the cinnamaldehyde content of cinnamon oil as high as 86 %, depending on their geographical origin (Ravishankar et al. 2009). Lemongrass oil contains multiple components including citral (57.5 %), citral diethylacetal (24.7 %), limonene (6.4 %), citral

acetate (2.1 %), myrcene (1.2 %), and methyl heptenone (1.2 %) (Katsukawa et al. 2010). Eugenol is the primary active component in both clove bud oil (up to 85 %) (Farang et al. 1989; Bauer et al. 2001) and allspice oil (Takemasa et al. 2009) and accounts for their antioxidant properties (Ogata et al. 2000; Takemasa et al. 2009).

Many phytochemicals are routinely used in the average domestic kitchen cabinet. Their longstanding usage has resulted in many of these antimicrobials being considered as Generally Regarded as Safe (GRAS) compounds (Dillon 1999; Ress et al. 2003; Adams et al. 2004; Knowles et al. 2005). Plant extracts/essential oils have been used in many commercial applications e.g., to provide flavoring to foods and fragrances in perfumes. They have also been added to toothpastes, shampoos, ointments, and cosmetics (Burt 2004). Oregano oil has been used in salad dressings, tomato sauces, and pizzas (Ravishankar et al. 2009). Lemongrass is widely used as a food flavoring and fragrance component in perfumes and also for its analgesic and anti-inflammatory characteristics (Ress et al. 2003; Katsukawa et al. 2010). Cinnamon oil is also used as a flavoring agent in some foods (Friedman et al. 2002).

1.1. Types of Plant Antimicrobials

There are several groups of plant antimicrobials including saponins, thiosulfinates, glucosinolates, terpenoids, and polyphenols. Saponins are naturally occurring glycosides that may exhibit antimicrobial, hemolytic, membrane depolarizing, cholesterol binding, and allopathic activities (Naidu 2000). Thiosulfinates are related to garlic, which has been used as a medicinal plant since antiquity and possesses both static and cidal effects against microbes (Naidu 2000). Glucosinolates are bioactive compounds found in cruciferous vegetables such as cabbage and broccoli (Naidu 2000). Terpenoids are secondary metabolites and are highly enriched in compounds based on an isoprene structure (Cowan 1999). Citral is a type of terpenoid that causes bacterial membrane disruption and the leakage of intracellular ions. Its action on the cell membrane also has dramatic effects on proton motive forces, intracellular ATP content, and the overall cell activity (Somolinos et al. 2010).

Phenolic compounds are present in all plants (Bravo 1998). To date, more than 8000 phenolic compounds have been identified. The phenols and polyphenols are a group of bioactive chemicals which consist of a single substituted phenolic ring (Cowan 1999); different compounds vary in their C₆ ring structure. In general, phenols or polyphenols exhibit the greatest antimicrobial efficacy of various plant origin compounds (Burt 2004). The antioxidant activity may occur via mechanisms such as scavenging of radicals and chelating of metal ions. The phenol, eugenol, reportedly participates in photochemical reactions (Mihara and Shibamoto 1982) and inhibits the production (Farang et al. 1989) and activity of enzymes (Wendakoon and Sakaguchi 1995). It may also cause changes in membrane permeability resulting from the exit of potassium ions (Walsh et al. 2003). Cell wall deterioration and lysis have also been observed (Thoroski et al. 1989).

Catechol and epicatechin are two forms of simple phenols (Peres et al. 1997; Toda et al. 1991). Phenolic acids, quinones, flavonoids, flavones, tannins (also called proanthocyanidins), and coumarins round out the class of phenolics. Red, blue, and purple berries, red and purple grapes, red wines, teas (especially green), apples, pears, raspberries, apples, onions, broccoli, soybeans, legumes, ginkgo biloba, and chocolate are common sources of polyphenols (Ullah and Khan 2008). Polyphenols exhibit anti-inflammatory, antimicrobial, anti-tumor, cardioprotective, and neuroprotective properties (Ullah and Khan 2008). Grape seed extract contains tannins, olive extract contains oleuropein, and green tea extract contains tannin and catechins. The high concentrations of epicatechin and catechin in grape seed extract and caffeic acid and epicatechin in green tea extracts account for their high antioxidant activities (Rababah et al. 2004).

Flavonoids are the most common polyphenolic compounds in plants (Kris-Etherton and Keen 2002) with over 4000 identified (Higdon and Drake 2005). They are found in numerous foods such as fruits (e.g., citrus, berries, apples), vegetables (e.g., onions), herbs, wines, teas, coffee, and chocolate (Higdon and Drake 2005). Flavonoids are considered secondary plant metabolites some of which are essential for plant function including roles in supportive tissues, plant defense strategies, and signaling properties (Garcia-Salas et al. 2010).

2. ANTIVIRAL ACTIVITY OF PLANT COMPOUNDS

While the antimicrobial properties of plant based essential oils have been examined in detail against bacteria (Didry et al. 1994; Friedman et al. 2002; Knowles et al. 2005; Peñalver et al. 2005; Callaway et al. 2008; Ravishankar et al. 2009; Reichling et al. 2009; Ravishankar et al. 2010), they have only recently been examined for their antiviral properties. The majority of this work has been directed towards enveloped viruses of clinical importance (Reichling et al. 2005; Koch et al. 2008; Loizzo et al. 2008; Meneses et al. 2009; Reichling et al. 2009; Garcia et al. 2010; Jackwood et al. 2010; Ocazonez et al. 2010; Wu et al. 2010; Garozzo et al. 2011), while limited research has been done on the efficacy of essential oils against non-enveloped viruses. In general, non-enveloped viruses are more resistant to environmental conditions and the action of antimicrobials than enveloped viruses (Watanabe et al. 1989; Barker et al. 2001).

It is impossible to describe every scientific paper examining the antiviral properties of plant compounds and hence the focus of this chapter is to provide the reader with examples of the types of studies that have been performed to date (e.g., the types of antimicrobials, the viruses tested); the results of such studies (e.g., whether or not the compound had antiviral activity against different types of viruses, namely viruses classified in different families or groups,

enveloped or non-enveloped viruses, DNA or RNA viruses, and viruses causing respiratory or gastrointestinal symptoms, etc.); and to point out any clues as to the possible mechanisms of antiviral action of plant antimicrobials.

The viruses mentioned in the cited research studies are listed in Table 16.1 along with their name abbreviations and the basic characteristics by which they may be generally categorized. This chapter has been divided into separate sections discussing enveloped and non-enveloped viruses since the mode of antiviral action, and thus the effectiveness of various plant antimicrobials, are likely to vary significantly between these two major virus types.

2.1. Efficacy of Plant Antimicrobials Against Enveloped Viruses

Numerous researchers have studied plant compounds and their antiviral effects against herpesviruses. Astani et al. (2011) investigated the antiviral efficacy of star anise essential oil (consisting of about 80 % of the phenylpropanoid, trans-anethole) as well as several other plant phenylpropanoids and sesquiterpenes (trans-anethole, eugenol, β -eudesmol, farnesol, β -caryophyllene and β -caryophyllene oxide) against herpes simplex virus type 1 (HSV-1). The antimicrobials were added to HSV-1 prior to cell infection and also after the viruses were already infecting cells. Star anise oil was the most effective, inhibiting viral infectivity by >99 %, followed by β -caryophyllene, a sesquiterpene (98 % inhibition). Trans-anethole and farnesol inhibited HSV-1 infectivity by >90 %. The other compounds reduced the cell infectivity by 60–90 %.

Koch et al. (2008) observed similar inhibitory effects of anise oil as well as oils from thyme, hyssop, ginger, chamomile, and sandalwood on herpes simplex virus type 2 (HSV-2). In another study, 12 essential oils (e.g., tea tree, cypress, juniper, tropical basil, peppermint, marjoram, eucalyptus, ravensara, lavender, lemon, rosemary, and lemongrass oils) were tested against HSV-1. A 1 % concentration of these oils completely inhibited virus infectivity but had no effect on intracellular virus. Lemongrass oil was the most effective, completely inhibiting virus infectivity at a concentration of 0.1 % (Minami et al. 2003). In a study by Armaka et al. (1999), a 1 % concentration of isoborneol, a monoterpenoid alcohol and a component of several plant essential oils, showed virucidal activity against HSV-1 ($\sim 4\text{-log}_{10}$ reduction in cell culture infectivity) within 30 min of exposure.

Other plant compounds including tea tree oil (Carson et al. 2001), eucalyptus oil (Schnitzler et al. 2001), Australian tea tree oil (Schnitzler et al. 2001), hyssop oil (Schnitzler et al. 2007), thyme oil (Schnitzler et al. 2007; Koch et al. 2008), manuka oil (Reichling et al. 2005), oregano oil (Siddiqui et al. 1996), ginger oil (Schnitzler et al. 2007), sandalwood oil (Schnitzler et al. 2007), carvacrol (Lai et al. 2012), thymol (Lai et al. 2012), eugenol (Lai et al. 2012), and menthol (Lai et al. 2012) have also been shown to have antiviral activity against herpesviruses. Crude extracts from *Guazuma ulmifolia* (mutumba) and *Stryphnodendron adstringens* stem bark (barbatimão) inhibited bovine herpesvirus type 1 (BHV-1) replication in infected cells (Felipe et al. 2006).

Table 16.1 Viruses referenced in this paper and their basic characteristics

<i>Virus Name</i>	<i>Abbreviation</i>	<i>External characteristic</i>	<i>Virus family</i>	<i>Viral genome</i>
Avian infectious bronchitis virus	AIBV	Enveloped	Coronaviridae	(+)ssRNA
Bovine herpesvirus type #	BHV-type #**	Enveloped	Herpesviridae	dsDNA
Bovine viral diarrhea virus	BVDV	Enveloped	Flaviviridae	(+)ssRNA
Dengue virus types 1–4	DENG-1, 2, 3, 4	Enveloped	Flaviviridae	(+)ssRNA
Herpes simplex virus types 1 & 2	HSV-1, HSV-2	Enveloped	Herpesviridae	dsDNA
Human cytomegalovirus	HCMV	Enveloped	Herpesviridae	dsDNA
Human immunodeficiency virus type 1	HIV-1	Enveloped	Retroviridae	ssRNA
Human respiratory syncytial virus	HRSV	Enveloped	Paramyxoviridae	(-)ssRNA
Influenza A virus	INFV-A	Enveloped	Orthomyxoviridae	(-)ssRNA
Influenza B virus	INFV-B	Enveloped	Orthomyxoviridae	(-)ssRNA
Junin virus	JUNV	Enveloped	Arenaviridae	(-)ssRNA
Measles virus	MeV	Enveloped	Paramyxoviridae	(-)ssRNA
Mumps virus	MuV	Enveloped	Paramyxoviridae	(-)ssRNA
Newcastle disease virus	NDV	Enveloped	Paramyxoviridae	(-)ssRNA
Ross River virus	RRV	Enveloped	Togaviridae	(+)ssRNA
SARS coronavirus	SARS-CoV	Enveloped	Coronaviridae	(+)ssRNA
Viral hemorrhagic septicemia virus	VHSV	Enveloped	Rhabdoviridae	(-)ssRNA
Yellow fever virus	YFV	Enveloped	Flaviviridae	(+)ssRNA
Adenovirus type #**	AdV-type #**	Non-Enveloped	Adenoviridae	dsDNA
Bovine rotavirus Group A	BRV-A	Non-Enveloped	Reoviridae	dsRNA
Coxsackie virus type #**	CV-type #**	Non-Enveloped	Picomaviridae	(+)ssRNA
Echovirus type #**	EV-type #**	Non-Enveloped	Picomaviridae	(+)ssRNA

(continued)

Table 16.1 (continued)

<i>Virus Name</i>	<i>Abbreviation</i>	<i>External characteristic</i>	<i>Virus family</i>	<i>Viral genome</i>
Feline calicivirus F9	FCV-F9	Non-Enveloped	<i>Caliciviridae</i>	(+)ssRNA
Fowl adenovirus	FAdV	Non-Enveloped	<i>Adenoviridae</i>	dsDNA
Hepatitis A virus	HAV	Non-Enveloped	<i>Picomaviridae</i>	(+)ssRNA
Human norovirus	HuNoV	Non-Enveloped	<i>Caliciviridae</i>	(+)ssRNA
Human rhinovirus type 2	HRV-2	Non-Enveloped	<i>Picomaviridae</i>	(+)ssRNA
Human rotavirus	RV	Non-Enveloped	<i>Reoviridae</i>	dsRNA
Murine norovirus type 1	MNV-1	Non-Enveloped	<i>Caliciviridae</i>	(+)ssRNA
Poliovirus type #	PV-type #*	Non-Enveloped	<i>Picomaviridae</i>	(+)ssRNA
Simian rotavirus SA-11	RV-SA-11	Non-Enveloped	<i>Reoviridae</i>	dsRNA
MS2 bacteriophage	MS2	Non-Enveloped	<i>Leviviridae</i>	(+)ssRNA
ΦX174 bacteriophage	ΦX174	Non-Enveloped	<i>Microviridae</i>	ssDNA
T4 bacteriophage	T4	Non-Enveloped	<i>Myoviridae</i>	dsDNA

*Abbreviation used in the current paper

#Have numerous types that are referred to in the text. The "type #" would be replaced in the actual name (or abbreviation). For instance, Cocksackie virus type B1 (CV-B1) and poliovirus type 1 (PV-1)

A potent antiviral activity against human immunodeficiency virus type 1 (HIV-1) and HSV-1 was observed inside of infected lymphocytes and macrophages *in vitro* using an aqueous extract from the South American medicinal plant *Baccharis trinervis* (Palomino et al. 2002). Garcia et al. (2010) evaluated seven essential oils from plants in Argentina against the following enveloped viruses: HSV-1, HSV-2, dengue virus type 2 (DENV-2), and junin virus (JUNV). The latter was the most resistant to essential oils. The most effective antiviral was essential oil from *Lantana grisebachii* against DENV-2, HSV-1, and HSV-2.

Ocazonez et al. (2010) found that essential oils from *Lippia alba* and *L. citriodora* were effective against DENV types 1 through 4 although oil from *L. alba* was more effective at lower concentrations than that from *L. citriodora*. These two essential oils, along with the oils from *Oreganum vulgare* and *Artemisia vulgaris*, were also shown to be effective against yellow fever virus (YFV; an enveloped virus) in a previous study (Meneses et al. 2009). *L. origanoides* oil at 11.1 µg/ml and *L. alba*, *O. vulgare* and *A. vulgaris* oils at 100 µg/ml produced 100 % reduction in the virus yield. Citral, a significant component of lemongrass oil, also exhibited antiviral activity against YFV and HSV-1 in previous studies (Astani et al. 2010; Gómez et al. 2013).

In another study (Pilau et al. 2011), Mexican oregano (*Lippia graveolens*) essential oil and its major component carvacrol were evaluated against several enveloped viruses including HSV-1 (acyclovir resistant and sensitive strains), human respiratory syncytial virus (HRSV), BHV-1, BHV-2, BHV-5, and bovine viral diarrhea virus (BVDV). BHV-1 and BHV-5 were not inhibited by the oregano essential oil, whereas the other five viruses were. Carvacrol was not effective against BHV-2. In addition, carvacrol was not as effective as oregano essential oil against other viruses in the study suggesting that some other component of oil was responsible for antiviral activity.

Yamada et al. (2009) determined that hydroxytyrosol, a small-molecule phenolic compound extracted from olive tree leaves, inactivated influenza A viruses (INFV-A) (H1N1, H3N2, H5N1, and H9N2 strains) and Newcastle disease virus (NDV) in both concentration and time-dependent manners. Olive leaf extracts have also been shown to be effective against HIV-1 (Bao et al. 2007; Lee-Huang et al. 2003) and viral hemorrhagic septicemia virus (VHSV) of fish (Micol et al. 2005). A sesquiterpene (triptofordin C-2) had moderate virucidal activity against several enveloped viruses including HSV-1, human cytomegalovirus (HCMV), measles virus (MeV), and INFV-A (H1N1 strain) (Hayashi et al. 1996). Siddiqui et al. (1996) found that oregano oil and clove oil were effective against enveloped viruses HSV-1 and NDV.

Two phytochemicals, betulinic acid and savinin, were shown to have antiviral activity against the SARS (severe acute respiratory syndrome) coronavirus (SARS-CoV) (Wen et al. 2007). Loizzo et al. (2008) studied the antiviral activity of essential oils obtained from berries and fruits of plant species in Lebanon. They found strong antiviral activity of *Laurus nobilis* oil (from berries) and

moderate antiviral activity of *Thuja orientalis* oil (from fruit) and *Juniperus oxycedrus* ssp. *oxycedrus* oil (from berries) against SARS-CoV. *Pistacia palaestina* essential oil (from fruit) was inactive against SARS-CoV.

2.2. Efficacy of Plant Antimicrobials Against Non-Enveloped Viruses

Several studies have included a comparison of the antiviral efficacy of plant antimicrobials against both enveloped and non-enveloped viruses. Not surprisingly, the observed antiviral effect has been greater for enveloped viruses in general. For instance, a sesquiterpene (triptofordin C-2) had moderate virucidal activity against several enveloped viruses (HSV-1, HCMV, MeV, and INFV-A), but was not effective against the non-enveloped poliovirus type 1 (PV-1) and Coxsackie virus type B1 (CV-B1) (Hayashi et al. 1996). Oregano oil and clove oil were effective against HSV-1 and NDV, but not against the non-enveloped PV-1 and Adenovirus type 3 (AdV-3) (Siddiqui et al. 1996). Hydroxytyrosol, a small-molecule phenolic compound extracted from olive tree leaves, inactivated INFV-A (H1N1, H3N2, H5N1, and H9N2 strains) and NDV, but was not effective against bovine rotavirus Group A (BRV-A) or fowl adenovirus (FAV), two non-enveloped viruses (Yamada et al. 2009).

Garozzo et al. (2009) examined tea tree oil and its main antimicrobial components, terpinen-4-ol (36.7 % of total oil), γ -terpinene (22.2 %), α -terpinene (10.1 %), ρ -cymene (2.5 %), terpinolene (3.5 %), and α -terpineol (2.7 %) against HSV-1, HSV-2, and INFV-A (subtype H1N1), as well as the following non-enveloped viruses: PV-1, echovirus type 9 (EV-9), CV-B1, and adenovirus type 2 (AdV-2). Tea tree oil, terpinen-4-ol, terpinolene, and α -terpineol had an inhibitory effect on INFV-A. Only a slight antiviral effect was observed for 0.125 % (vol/vol) tea tree oil against HSV-1 and HSV-2. None of the antimicrobials was effective against any of the non-enveloped viruses. In another study (Pilau et al. 2011), Mexican oregano (*Lippia graveolens*) essential oil and its major component carvacrol were evaluated against the enveloped viruses HSV-1 (acyclovir resistant and sensitive strains), HRSV, BHV-1, BHV-2, BHV-5, and BVDV. Human rotavirus (RV; non-enveloped) was also included in the study. RV, BHV-1, and BHV-5 were not inhibited by the oregano essential oil, whereas the other five viruses were inhibited. Carvacrol alone was effective against RV but not against BHV-2.

In contrast, Semple et al. (2001) showed that chrysophanic acid (an anthraquinone) from the Australian Aboriginal medicinal plant *Dianella longifolia* inhibited poliovirus-induced cytopathic effects in BGMK (Buffalo green monkey kidney) cells for poliovirus types 2 and 3 (PV-2 and PV-3); however, it did not have significant antiviral activity against two enveloped viruses, HSV-1 and Ross River virus (RRV), or against related non-enveloped enteroviruses: Coxsackie virus types A21 and B4 (CV-A21 and CV-B4, respectively) and human rhinovirus type 2 (HRV-2).

In recent studies, non-enveloped viruses have been studied separately from enveloped viruses. For example, Búfalo et al. (2009) studied the plant *Baccharis dracunculifolia* (extract and essential oil), Brazilian propolis (the resinous substance produced by honeybees from this plant), and caffeic and cinnamic acids against PV-1. The extract worked better than the essential oil and they both worked better than the individual components (caffeic and cinnamic acids). In a study by Tait et al. (2006), homoisoflavonoids were evaluated against several enteroviruses. None were effective against PV-1 but all had marked antiviral activity against CV-B1, CV-B3, CV-B4, and CV-A9, and echovirus type 30 (EV-30).

In the last few years, there has been renewed interest in examining plant antimicrobials against viral foodborne pathogens or their surrogates. Su et al. (2010a) examined the efficacy of cranberry juice and cranberry proanthocyanidins (PAC) on enteric virus surrogates: murine norovirus type 1 (MNV-1), feline calicivirus F9 (FCV-F9), MS2 (a ssRNA bacteriophage), and Φ X174 (a ssDNA bacteriophage). MNV-1 was reduced by 2.06- \log_{10} PFU/ml with cranberry juice, and 2.63- \log_{10} PFU/ml with 0.15 mg/ml PAC. FCV-F9 was undetectable after exposure to both cranberry juice and 0.15 mg/ml PAC. MS2 titers were reduced by 1.14- \log_{10} with cranberry juice and by 0.96- \log_{10} PFU/ml with 0.60 mg/ml PAC. Φ X174 titers were reduced by 1.79- \log_{10} with cranberry juice and by 4.98- \log_{10} PFU/ml with 0.60 mg/ml PAC. Neutralizing the pH of the cranberry juice did not diminish the antiviral effect.

Su et al. (2011) also evaluated pomegranate juice and pomegranate phenolic extracts (PPE) against FCV-F9, MNV-1, and MS2 phage. After 20 min of exposure to pomegranate juice, reductions of 3.1- and 0.8- \log_{10} of FCV-F9 and MNV-1 infectivity, respectively, were observed. A 4 mg/ml concentration of PPE resulted in slightly higher reductions of 5.1- and 1.1- \log_{10} of the two viruses, respectively, following 20 min of exposure. Neither pomegranate juice nor PPE was effective against MS2 phage after 20 min.

Elizaquível et al. (2013) studied the antiviral efficacy of clove, oregano, and zataria essential oils against the human norovirus (HuNoV) surrogates, FCV-F9 and MNV-1 at 4 and 37 °C. Concentrations of 2 % oregano oil, 1 % clove oil, and 0.1 % zataria oil were less effective at 4 °C than at 37 °C. At 4 °C, MNV-1 titers were reduced by 0.6-, 0.8-, and 1.0- \log_{10} , respectively, while reductions of 1.6-, 0.7-, and 0.3- \log_{10} were observed at 37 °C. The oils were not effective against FCV-F9 at 4 °C (≤ 0.25 - \log_{10} reduction) but were highly effective at 37 °C (3.8-, 3.8-, and 4.5- \log_{10} reductions for oregano, clove, and zataria oils, respectively).

Gilling et al. (2014a) examined the effects of oregano oil and its primary antimicrobial component, carvacrol, against MNV-1. Both antimicrobials caused statistically significant reductions ($P \leq 0.05$) in cell culture infectivity within 15 min of exposure (~ 1.0 - \log_{10}). Despite this, the MNV-1 infectivity remained stable over time with exposure to oregano oil (1.07- \log_{10} after 24 h), while carvacrol was far more effective, producing up to 3.87- \log_{10} reductions

within 1 h of exposure and $>4.15\text{-log}_{10}$ after 3 h. In a separate study, allspice oil, lemongrass oil, and citral (a component of lemongrass oil) were moderately effective against MNV-1, producing significant reductions of $0.9\text{--}1.88\text{-log}_{10}$ after 6 h (Gilling et al. 2014b).

The antiviral effects of grape seed extract were studied against the food-borne pathogen hepatitis A virus (HAV; non-enveloped) and the surrogates FCV-F9, MNV-1, and MS2 bacteriophage (Su and D'Souza 2011). FCV-F9 was significantly reduced (in a dose-dependent manner) by up to 4.98-log_{10} PFU/ml, MNV-1 by up to 1.97-log_{10} PFU/ml, MS2 by up to 1.85-log_{10} PFU/ml, and HAV up to 2.89-log_{10} PFU/ml after treatment at 37°C with grape seed extract. Comparable reductions of up to 5.01- , 1.67- , 1.16- , and 3.01-log_{10} were observed for the four viruses, respectively, with grape seed extract at room temperature.

In a study by Li et al. (2012), grape seed extract was effective against non-enveloped human norovirus GII.4 (HuNoV GII.4) and MNV-1. There was a reduction in MNV-1 infectivity by $>3.0\text{-log}_{10}$, and in HuNoV GII.4 specific binding to Caco-2 cells by $>1.0\text{-log}_{10}$ genomic copies/ml (as determined by real time quantitative RT-PCR) following treatment. The anti-MNV-1 activity was quite limited for surface disinfection ($<1\text{-log}_{10}$ PFU/ml after a 10-minute exposure on stainless steel discs), but a $1.5\text{--}2\text{-log}_{10}$ PFU/ml reduction in MNV-1 cell culture infectivity was observed with 2 mg/ml of grape seed extract used for a sanitizing wash water for fresh-cut iceberg lettuce. The grape seed extract was effective regardless of the chemical oxygen demand ($0\text{--}1500$ mg/ml) of the wash water.

3. MECHANISMS OF ANTIVIRAL ACTION

Due to the numerous components found in most essential oils, they often have multiple effects on the bacterial cell (Burt 2004). They may cause deterioration of the cell wall (Thoroski et al. 1989; Burt 2004), damage to cell membranes (Ultee et al. 2002) and cell membrane proteins (Ultee et al. 1999), increased membrane permeability and leakage of cell contents (Ultee et al. 2002; Burt 2004), coagulation of cytoplasm (Gustafson et al. 1998), reduction of proton motive force (Ultee et al. 1999), inactivation of critical enzymes (Wendakoon and Sakaguchi 1995; Cowan 1999; Ayala-Zavala et al. 2008), and disturbance of genetic material functionality (Ayala-Zavala et al. 2008).

The mechanisms of antiviral action of plant antimicrobials have not been studied in detail and thus are not well understood. Due to the differences between bacterial cells and virus particles (both enveloped and non-enveloped), the mechanisms are likely quite different. Nevertheless, some overlap may occur. For instance, antimicrobials that act on the outer phospholipid membrane of Gram-negative bacteria may also be active against the envelope of some viruses. In addition, antimicrobials which act on proteins or on nucleic acid may cause similar effects in both classes of microorganisms.

It is difficult to determine if reductions in virus infectivity are due to direct damage to the virus particles or to a simple inhibition of virus adsorption to the host cells. The viral inactivation might not even require a metabolic process. For instance, the virus may be immobilized to a surface, the antimicrobial could cause the virus particles to clump, the host-cell receptors may be blocked by the antimicrobial bound to virus surfaces, viral replication may be inhibited, or the nucleic acid within the viral capsid may be inactivated (Thurman and Gerba 1989). The presence or absence of a viral envelope is likely to play a significant role in the effectiveness (or lack thereof) of the antimicrobial since the exterior surface of the virus is what makes first contact with the antimicrobial.

3.1. Mechanisms of Antiviral Activity Against Enveloped Viruses

To date, the antiviral mechanisms of action for plant essential oils and other components have not been adequately evaluated. The majority of this work has been performed with enveloped viruses and with clinical treatments in mind; therefore, the focus has been on the inhibition of viral adsorption to host cells or viral uptake into the cells by plant antimicrobials or on examining their effectiveness against already intracellular viruses.

In a study of star anise essential oil and several plant phenylpropanoids and sesquiterpenes, Astani et al. (2011) found that the plant antimicrobials worked on HSV-1 directly and had no antiviral effect ($P \geq 0.2$) once the virus was inside the cell. The authors speculated that the plant compounds either: (1) inactivated the virus directly, (2) interfered with the virus envelope, or (3) blocked viral structures that are necessary for virus adsorption or entry into host cells. Koch et al. (2008) also found similar results for cell infectivity with oils from anise, thyme, hyssop, ginger, chamomile, and sandalwood with HSV-2. In addition, they found that pretreatment of the host cells with essential oils did not prevent viral infection.

Minami et al. (2003) reported that 12 essential oils (tea tree, cypress, juniper, tropical basil, peppermint, marjoram, eucalyptus, ravensara, lavender, lemon, rosemary, and lemongrass oils) completely inhibited HSV-1 cell culture infectivity but had no effect on intracellular viruses. Isoborneol, a component of several plant essential oils, caused approximately a 4- \log_{10} reduction in HSV-1 cell culture infectivity and specifically inhibited the glycosylation of viral proteins but did not appear to prevent virus adsorption to the cells (Armaka et al. 1999). Likewise, the plant sesquiterpene triptofordin C-2 did not appear to prevent HSV-1 adsorption to host cells (Hayashi et al. 1996). Two phytochemicals, betulinic acid and savinin, were shown to have antiviral activity against the SARS-CoV. Both compounds exhibited protease inhibition and appeared to inhibit post-binding entry of the virus into cells (Wen et al. 2007).

Ocazonez et al. (2010) found that exposure to essential oils from *L. alba* and *L. citriodora* was effective against DENV when applied 2 h prior to cell adsorption, but no effect was observed for viruses already located within the

host cells. In another study, essential oils from *L. alba*, *L. origanoides*, *O. vulgare*, and *A. vulgaris* were effective against YFV; however, no reductions were observed when the cells were treated with essential oils prior to virus adsorption (Meneses et al. 2009) indicating that a different antimicrobial mechanism was operating against this virus. The essential oil from Mexican oregano (*Lippia graveolens*) inhibited HSV-1 (acyclovir resistant and sensitive strains), HRSV, and BHV-2 both before and after virus inoculation while carvacrol (its primary active component) caused inhibition only after virus inoculation (Pilau et al. 2011).

Jackwood et al. (2010) treated avian infectious bronchitis virus (AIBV) (an enveloped coronavirus) with oleoresins and essential oils from botanicals in a liquid emulsion product [QR448(a)]. The anti-AIBV activity was greater prior to virus attachment, indicating that the effect is likely directly virucidal; however, treatment of the chickens prevented symptoms but not infection for 4 days following treatment and also decreased the transmission of the virus (Jackwood et al. 2010).

In relatively few studies, the antimicrobials have been found effective against intracellular viruses. For instance, antiviral activity against HIV-1 and HSV-1 was observed inside infected lymphocytes and macrophages *in vitro* using an aqueous extract from the South American medicinal plant *Baccharis trinervis*. The effects appeared to take place primarily in the early steps of virus replication e.g., during virus-cell attachment, virus-cell fusion, and cell-to-cell fusion (Palomino et al. 2002). Garozzo et al. (2011) determined that tea tree oil and its components worked against influenza virus type A only when added within 2 h of infection. Only a slight reduction was observed when they were added during virus adsorption to the cells. The tea tree oil and its components did not interfere with virus adsorption to host cells. In addition, the treatment of infected cells with 0.01 % (vol/vol) of tea tree oil before staining seemingly inhibited viral uncoating by interfering with the acidification of the intralysosomal compartment.

Green tea extract and one of its components, epigallocatechin, was found to inhibit the acidification of endosomes and lysosomes in influenza A and influenza B infected cells in another study. The growth of these viruses was also inhibited if the cells were treated with green tea extract as early as 5 min following virus infection (Imanishi et al. 2002). Similar results were observed with the extract of *Ephedrae herba*, which inhibited the acidification of endosomes and lysosomes in cells infected with influenza A virus in a concentration-dependent manner. The growth of the virus was inhibited when cells were treated within 5–10 min following infection (Mantani et al. 1999).

Wu et al. (2010) studied the effect of On Guard™, a commercial blend of oils from wild orange, clove, cinnamon, eucalyptus, and rosemary, on H1N1 influenza virus. The treatment did not affect virus adsorption or internalization into host cells, but did result in a loss in cell culture infectivity. The treated viruses continued to express viral mRNAs but had reduced expression of viral proteins, indicating that the essential oil blend likely inhibits viral protein synthesis.

Similarly, *trans*-cinnamaldehyde inhibited influenza A viral protein but not mRNA synthesis in a previous study (Hayashi et al. 2007).

Yamada et al. (2009) determined that hydroxytyrosol, a small-molecule phenolic compound extracted from olive tree leaves, did not prevent INFV-A strain H9N2 binding to host cells, but viral mRNA and proteins were absent once the virus entered the cells. The virus also appeared to be physically altered under transmission electron microscopy (TEM), with ill-defined structure and the apparent loss of surface protein spikes. Similarly, Siddiqui et al. (1996) observed apparent dissolution of the viral envelopes of HSV-1 and NDV with both oregano and clove oil under TEM. Lai et al. (2012) also observed what appeared to be holes via TEM in the HSV-1 envelope following treatment with carvacrol and thymol. The following mechanisms appear to be the most relevant for enveloped viruses:

- (1) Direct virucidal effect on the enveloped virus reducing the ability of the virus to infect host cells. Such activity does not appear to prevent virus adsorption to host cells but may include dissolution of the viral envelope or proteolytic activity that acts on virus spike proteins and receptor proteins, preventing viral entry into host cells.
- (2) Inhibition of the early stages (within a few hours) of virus replication within the cells. There is apparently no (or little) effect on intracellular viruses after this time period.
- (3) Inhibition of viral uncoating by reducing acidification of endosomes and lysosomes in the host cell.
- (4) Inhibition of viral protein synthesis or viral protein modifications (e.g., glycosylation) inside the host cell.

It should be noted that some antimicrobials may exhibit more than one of these effects against specific enveloped viruses.

3.2. Mechanisms of Antiviral Activity Against Non-Enveloped Viruses

Recent studies have attempted to elucidate the mechanisms of action of plant antimicrobials (essential oils and extracts or their components) against non-enveloped viruses. The protein capsid in non-enveloped viruses serves to protect the viral nucleic acid and to initiate infection by facilitating virus adsorption to the host cells (Cliver 2009). In a review by Cliver (2009), it was found that antimicrobials and other treatments that inactivate small enteric viruses [caliciviruses (e.g., HuNoV, MNV-1, and FCV-F9), picornaviruses (e.g., PV-1, EV-9, CV-B1), hepatitis viruses (e.g., HAV), and astroviruses], act on the virus capsid to some extent; in many cases, the viral RNA is unaffected though the virus is no longer infectious.

Búfalo et al. (2009) studied the effects of plant *Baccharis dracunculifolia* (extract and essential oil), Brazilian propolis (the resinous substance produced by honeybees from this plant), and caffeic and cinnamic acids against PV-1. They found that treatment with the antimicrobials at the same time as

exposure to the host cells was more effective than treatment after the viruses were inside the cells. In a study by Semple et al. (2001), chrysophanic acid (an anthraquinone) from the Australian Aboriginal medicinal plant *Dianella longifolia* inhibited PV-2 and PV-3 induced cytopathic effects in BGM cells. The antimicrobial appeared to work best when added during or immediately following virus adsorption to the cells, suggesting that it acted early during the poliovirus replication cycle. In a study by Felipe et al. (2006), crude extracts from *Guazuma ulmifolia* (mutumba) and *Stryphnodendron adstringens* stem bark (barbatimão) inhibited PV-1 replication in infected cells. In another study, T4 bacteriophage was incapable of adsorbing to its bacterial host cells after exposure to cranberry juice (as visualized under scanning and transmission electron microscopy) (Lipson et al. 2007).

In a study by Cermelli et al. (2008), eucalyptus essential oil did not affect adenovirus but did cause a small reduction in cell infectivity of mumps virus (MuV). However, these researchers examined the effect of essential oil only after the viruses were inside the host cells. Based on the results for both non-enveloped and enveloped viruses in other studies, the antiviral activity of plant antimicrobials is more likely to occur prior to the internalization of the virus in the host cells. Therefore, any antiviral activity of eucalyptus oil may have been missed in this study.

Evidence of direct virucidal activity of plant antimicrobials on virus particles may be found in several published studies. Under TEM, HuNoV GII.4 virus-like particles treated with grape seed extract exhibited clumping, inflation (to approximately twice the original size), and deformation. With greater doses of grape seed extract, a large amount of protein debris was observed leading the authors to believe that this was due to direct damage to the virus capsid (Li et al. 2012). Treatment of FCV-F9 with cranberry juice and cranberry proanthocyanidins revealed structural changes in virus particles; they appeared to be damaged under TEM (Su et al. 2010b). Lipson et al. (2007) observed that rotavirus SA-11 (RV-SA11) treated with 20% cranberry juice had single-shelled or anomalous virus-like particles, rather than the double-shelled, icosahedral “wheel-like” particles found in the untreated control samples, indicating that cranberry juice may be involved in modification of rotavirus glycoprotein spike moieties.

Although significant reductions in cell culture infectivity of MNV-1 were observed following treatment with oregano oil and carvacrol, the virus adsorption to host cells did not appear to be affected (Gilling et al. 2014a). Based on an RNase I protection assay (which indicated that the virus capsid was no longer completely intact following antimicrobial exposure), oregano oil and carvacrol appeared to exert a virucidal effect directly upon the virus capsid and, subsequently, the exposed RNA. Under TEM, the MNV-1 capsids expanded from ≤ 35 nm in diameter to up to 75 nm following exposure to oregano oil and up to 800 nm following exposure to carvacrol; with greater expansion, capsid disintegration could be readily observed (Gilling et al. 2014a). In a separate study, the MNV-1 capsid expanded to approximately 75 nm in

diameter following exposure to allspice oil, which also appeared to act directly upon the virus capsid (based on an RNase I protection assay) (Gilling et al. 2014b).

In studies of lemongrass oil and its major active component, citral, against MNV-1, novel mechanisms appear to play a role in their antimicrobial efficacy (Gilling et al. 2014b). Both antimicrobials appeared to coat the MNV-1 capsid, causing indiscriminate binding of the virus particles to both the host cells and the plastic of the cell culture plates. The virus capsid and the RNA genome were seemingly completely intact; however, there was a significant reduction in virus cell culture infectivity.

Based on these limited studies, the following mechanisms appear to be relevant for some non-enveloped viruses:

- (1) Direct virucidal activity that reduces the ability of the non-enveloped virus to infect host cells. This would include the possible degradation of the virus capsid or protein spikes. Expansion/inflation of the virus capsid may occur which may in turn also lead to capsid disintegration. Such activity does not appear to prevent virus adsorption to host cells.
- (2) Denaturation/degradation of viral nucleic acid once exposed to the antimicrobial following capsid break down.
- (3) Antimicrobial coating of virus particles, leading to indiscriminate (non-specific) binding to host cells and the inhibition of cell infectivity. Capsid and viral nucleic acid are still intact.
- (4) Inhibition of the early stages (within a few hours) of virus replication within the host cells. There is apparently no (or little) effect on intracellular viruses after this time period.

Some antimicrobials may exhibit more than one of these effects against specific non-enveloped viruses.

4. CONCLUSIONS

Many plant essential oils, extracts, and individual chemical components have been demonstrated to possess antiviral efficacy against enveloped and/or non-enveloped viruses. In general, plant antimicrobials exhibit greater antiviral efficacy against enveloped viruses than non-enveloped viruses (though not in all cases). There appear to be multiple mechanisms of antiviral action for plant antimicrobials; nevertheless, the majority of antimicrobials appear to act either directly on the virus itself (e.g., on the envelope or capsid) or during the early stages of virus replication following internalization of the virus into its host cell.

Although numerous studies have been conducted with enveloped viruses, few are available on non-enveloped viruses that are more likely to be the causative agents of foodborne disease. Thus, our understanding of the mechanisms

of antiviral activity against non-enveloped viruses is incomplete. Future studies should, therefore, focus on determining the effectiveness of various plant antimicrobials against enteric foodborne viruses to address such data gaps and to attempt to further elucidate the antiviral mechanisms of action.

A great challenge in employing plant essential oils/extracts/components as food sanitizers is the problem of compatibility with respect to odor and taste. Regardless of the source, phytochemicals often have specific aromatic properties and it is important, therefore, to pair such plant antimicrobials with compatible food items, much like how wine and cheese are paired. For example, green tea extract could be used with lemons, limes, apples, and other fruits traditionally combined with both hot and cold tea drinks. Grape seed extract could be used on grapes, strawberries, raspberries, and other vine-based fruits. Many essential oils are derived from food products such as garlic and oregano that are commonly used in cuisines worldwide. Additionally, spices such as cinnamon and allspice are common flavor additives used in foods. Efforts should be made to determine optimal olfactory/organoleptic combinations and to determine the lowest effective antimicrobial concentrations. This would minimize the aromatic and sensory effects and lower the costs of such food treatments.

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