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Antimicrobial Activities of Essential Oils, Plant Extracts and their Applications in Foods- A Review

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Abstract

Food crops are subjected to contact with several foodborne pathogens that may be found in the surrounding soil, air and water sources and even fecal material that may be present in the field conditions. Improper processing and handling, temperature irregularities during transportation and storage, and inadequate sanitation options can also lead to foodborne outbreaks associated with frequently consumed foods. The ability of these pathogens to survive the processing and supply chain and reach consumers, causing outbreaks, has made interventions important in terms of sanitation measures. Natural antimicrobials such as plant essential oils and extracts are gaining popularity as an alternative to commercially used chemicals such as chlorine and hydrogen peroxide. Consumers are more aware of the harmful effects of these chemicals and prefer natural alternatives. Essential oils have been used as flavorings and perfumery agents for a long time, and have recently gained popularity in foods due to their antimicrobial activity. This manuscript aims to establish a comprehensive review on the antimicrobial activity of some plant essential oils, their active components and plant extracts against common foodborne pathogens *in vitr*o and on/in foods. Even though the exact mechanism of action may be unknown, the efficacy of these compounds in reducing the survival of pathogens, makes them prime candidates for alternate sanitizers.

Keywords: Essential oils, plant extracts, foodborne pathogens, antimicrobial activity, edible films

Introduction

Plant essential oils (EOs) and extracts have been long known to have beneficial properties but have recently been investigated for their use as antimicrobials. Essential oils and plant extracts are derived from various types of plant material such as the flowers, seeds, bark, wood, buds, fruits, and roots. These oils and extracts are obtained through various methods such as fermentation, extraction, expression, or enfleurage (Burt, 2004). Their active components can also be produced synthetically for commercial use (Burt, 2004; Cowan, 1999; Nazzaro et al., 2013). They can be composed of over 60 different components with particular compounds comprising up to 85% of the oil or extract, whereas others are only present in very small amounts (Burt, 2004; Nazzaro et al., 2013). Compounds found in EOs and extracts include phenols, polyphenols, terpenoids, flavonoids, flavones, flavonols, tannins, quinones, coumarins, alkaloids, lectins, and polypeptides (Cowan, 1999). According to Burt (2004), these compounds can be analyzed using techniques such as chromatography and mass spectrometry.

For centuries, EOs and extracts have been used for their aromatic nature with applications in perfumes and as flavor enhancers (Elgayyar et al., 2001). In addition, a number of EOs are currently in use for their preservative properties. For instance, "Protecta One" and "Protecta Two" are made in the US and are composed of an herbal blend of extracts suspended in solutions of sodium citrate and sodium chloride, respectively (Cutter, 2000).

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Another product is known as "DMC Base Natural" which is produced in Spain and is composed of 50% EOs from citrus, sage, rosemary, and 50% glycerol. Synergistic activity has also been observed against specific microorganisms when using EOs, extracts, and their components with common preservatives such as sodium chloride, sodium nitrate, and nisin (Burt, 2004). In addition, there is a vast amount of literature supporting the antioxidant and anti-inflammatory activities of EOs and extracts (Nazzaro et al., 2013). Furthermore, they are generally recognized as safe (GRAS) by the Food and Drug Administration in the US (Moreira et al., 2005). EOs, plant extracts, or their active components have been recognized for their antiviral, antimycotic, antiparasitic, insecticidal, antitoxigenic, and antibacterial properties (Burt, 2004; Cowan, 1999; Shan et al., 2007; Nazzaro et al., 2013). They even have promising activity against a number of antibiotic resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *Salmonella enterica* (Warnke et al., 2009; Ravishankar et al., 2010).

The antimicrobial activity of a compound can be influenced by its composition and extraction method, but also the volume of inoculum present, concentration of the EO or extract, the type and pH of media used, the growth phase of the organism, the use of an emulsifier or solvent to aid in suspension, as well as incubation times and temperatures (Nazzaro et al., 2013; Burt, 2004). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), which are determined using various methods such as disk diffusion, agar or broth dilutions, time kill assays or survival curves, and SEM are used to define the antimicrobial activity against a particular organism (Burt, 2004). It has been concluded by a number of studies that Gram-negative bacteria are less susceptible to the antimicrobial action of EOs and extracts than Gram-positive organisms due to the presence of a protective outer membrane; however, a number of studies have shown various EOs to be effective on Gram-negative bacteria (Nazzaro et al., 2013; Burt, 2004; Bajpai et al., 2012; Kim et al., 2011). *Pseudomonas aeruginosa* for example, has been found to be more resistant to EOs and extracts (Burt, 2004).

Antimicrobial activity against foodborne microorganisms

Many studies have demonstrated the strong *in vitro* antimicrobial activity of EOs and extracts against a wide range of Gram-positive and Gram-negative foodborne pathogens and spoilage bacteria (Gutierrez et al., 2008). Friedman et al. (2002) investigated the bactericidal activity of 96 EOs and 23 active components using the micro plate assay against *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Listeria monocytogenes,* and *S. enterica.* Twenty-seven oils and 12 active components were effective against all four bacterial species. *C. jejuni* was most susceptible to ginger root, gardenia, cedarwood, marigold, jasmine, patchouli, carrot seed, celery seed, mugwort, spikenard, orange bitter oils, as well as the active components cinnamaldehyde, estragole, carvacrol, benzaldehyde, citral, thymol, eugenol, perillaldehyde, R-(-)-carvone, and geranyl acetate. *E. coli* O157:H7 was most sensitive to oregano, thyme, cinnamon, palmarose, bay leaf, clove bud, lemongrass, and allspice oils, as well as the active components carvacrol, cinnamaldehyde, thymol, eugenol, salicylaldehyde, geraniol, isoeugenol, citral, perillaldehyde, and estragole (Friedman et al., 2002). *L. monocytogenes* was most sensitive to gardenia, cedarwood, bay leaf, clove bud, oregano, cinnamon, all spice, thyme, and patchouli oils, as well as the active components cinnamaldehyde, eugenol, thymol, carvacrol, citral, geraniol, perillaldehyde, S-(+)-carvone, estragole, and salicylaldehyde (Friedman et al., 2002). Lastly, *S. enterica* was most sensitive to thyme, oregano, cinnamon, clove bud, all spice, bay leaf, palmarose, and marjoram oils, as well as the active components thymol, cinnamaldehyde, carvacrol, eugenol, salicylaldehyde, geraniol, iso-eugenol, terpineol, perillaldehyde, and estragole (Friedman et al., 2002).

Shan et al. (2007) tested 46 different extracts from spices and medicinal herbs for antimicrobial activity against *Bacillus cereus*, *L. monocytogenes*, *Staphylococcus aureus*, *E. coli,* and *S.* Anatum.

Twelve out of the 46 extracts including shiliupo, oregano, cinnamon, clove, diyu, huzhang, and cassia with a high phenol content showed antimicrobial activity against the five foodborne pathogens with *S. aureus* being the most sensitive and *E. coli* being the most resistant.

Fisher & Phillips (2006) demonstrated the effectiveness of lemon, orange, and bergamot EOs and their active components against *C. jejuni*, *E. coli* O157:H7, *L. monocytogenes, B. cereus,* and *S. aureus in vitro.* They found bergamot, citral, and linalool to be the most effective. In addition, Elgayyar et al. (2001) evaluated the antimicrobial activity of anise, angelica, basil, carrot, celery, cardamom, coriander, dill weed, fennel, oregano, parsley, and rosemary EOs against a broader range of bacterial species. These included *L. monocytogenes, S. aureus, E. coli* O157:H7, *Yersinia enterocolitica, P. aeruginosa, Lactobacillus plantarum, Aspergillus niger,* and *Geotrichum rhodotorula.* Oregano, basil, and coriander EOs showed high inhibitory activity against the bacteria and fungi. Anise EO was not effective against bacteria but was effective

against the mold species. Furthermore, oregano oil has also been found to be effective against *Helicobacter pylori* (Fisher & Phillips, 2006).

Understanding the mechanism of action (MOA) for plant-based antimicrobials with varying compositions may aid in evaluating their potential application in complex food systems (Fitzgerald et al., 2004; Gill & Holley, 2004; Burt, 2004; Bajpai et al., 2010; Shan et al., 2007; Picone et al., 2013; Hyldgaard et al., 2012). However, when evaluating a potential antimicrobial agent, it is fairly easy to determine its range of activity or its structure, but determining the MOA can be a bit more challenging. Many studies have been performed, but precise mechanisms remain unknown (Nazzarro et al., 2013; Lambert et al., 2001). Since EOs and extracts are comprised of numerous compounds, it is suggested that EOs and extracts most likely have multiple targets resulting in primary MOAs and secondary MOAs instead of having one primary MOA that is attributed to one component (Carson et al., 2002; Burt, 2004; Nazzaro et al., 2013; Picone et al., 2013). This can be supported by the fact that active components or their mixtures were not as effective as the EO or extract as a whole, which implies that other minor compounds could play a role in the MOA (Burt, 2004).

Researchers have suggested that the primary MOA of EOs and extracts is related to the hydrophobic nature of the various components, which allows them to partition in the lipids of cell membranes, ultimately rendering them more permeable. Cells can typically tolerate a certain amount of leakage without losing viability, but if crucial amounts are lost, viability will also be lost (Burt, 2004; Nazzaro et al., 2013; Hyldgaard et al., 2012; Devi et al., 2010). In addition, Ultee et al. (2002) suggest that the phenolic ring itself, which is found in many EO and plant extract compounds, is attributed to the antimicrobial activity. This was concluded after evaluating other compounds that lack the phenolic ring, such as menthol, which had significantly reduced antimicrobial activity when compared to compounds found in EOs and extracts that contain the ring. Other studies have also stated a correlation between phenolic content and antimicrobial activity (Burt, 2004; Shan et al., 2007). The antimicrobial activities of a few commonly used EOs and plant extracts have been studied and reviewed as follows:

1.0 Clove Bud Oil (CBO)-Antimicrobial Activity

Clove bud oil (CBO) is derived from the buds of *Eugenia caryophyllata*, which is also known as *Syzygium aromaticum*. As stated previously, the composition of various EOs may differ depending on the extraction method as well as the plant itself. Burt (2004) stated that CBO is 75-85% eugenol and 8-15% eugenyl acetate. Chaieb et al. (2007) used gas chromatography-mass spectrometry (GC-MS) analysis and found that their particular CBO was comprised of 88.5% eugenol, 5.62% eugenyl acetate, 1.39% β-caryophyllene, and less than 1% of 2-heptanone, ethyl hexanoate, humulenol, α-humulene, calacorene, and calamenene. Fu et al. (2007) also used GC-MS and found the composition of their CBO to be comprised of 68.52% eugenol, 19% β-caryophyllene, 10.15% 2-methoxy-4-(2-propenyl)-phenol acetate, and 1.85% α-caryophyllene. Lastly, Du et al. (2009) found the composition of their CBO to be 81.58% eugenol, 11.53% eugenol acetate, 5.12% β-caryophyllene, and 0.56% α-humulene.

Studies have shown CBO to be effective against a wide range of bacteria commonly associated with foodborne illness as well as having antifungal, antiallergic, antimutagenic, anticarcinogenic, and antioxidant properties (Chaieb et al., 2007). CBO has demonstrated significant inhibitory activity against *S. aureus, E. coli, L. monocytogenes,* and *S. enterica* (Friedman et al., 2002; Shan et al., 2007).

Moreira et al. (2005) and Fisher & Phillips (2006) showed that CBO had notable bacteriostatic and bactericidal activity against various strains of *E. coli.* Nascimento et al. (2000) tested the extracts of yarrow, clove, lemon balm, basil, guava, pomegranate, rosemary, sage, jambolan, and thyme against *S. aureus, S.* Choleraesuis*, P. aeruginosa, B. subtilis, C. albicans, Proteus spp., Klebsiella pneumoniae, Enterobacter aerogenes*, and *E. coli.* The greatest antimicrobial activity was observed with the clove extract, which inhibited 57.1% of the organisms tested including both Gram-positive and Gram-negative bacteria. In addition, CBO was effective against 83.3% of the antibiotic resistant bacteria and *P. aeruginosa,* a bacterium known to be very resistant to the effects of EOs.

Warnke et al. (2009) also demonstrated that CBO had considerable activity against various hospital acquired antibiotic resistant strains of bacteria such as MRSA. Furthermore, Nuñez & D'Aquino (2012) used 0.4% CBO at 21°C to reduce *E. coli, S. aureus,* and *P. aeruginosa* populations by 5 log CFU/ml *in vitro.* They also tested the efficacy of 0.4% CBO at 21°C when influenced by organic matter using sterile rabbit serum, Brewer's yeast, and bovine serum albumin in distilled water. Their results showed that the organic matter did reduce the antimicrobial activity, but still maintained

bactericidal activity. Ayoola et al. (2008) performed antioxidant screening of CBO and found that eugenol, the active component of CBO, acted as a free radical scavenger.

The efficacy of CBO has also been evaluated using food models, incorporation into edible films, and for extending shelf-life. For instance, CBO had high antimicrobial activity against foodborne pathogens in yogurt and cucumber (Bajpai et al., 2012). Du et al. (2009) demonstrated that CBO reduced populations of *E. coli* O157:H7, *S. enterica,* and *L. monocytogenes* when incorporated into apple based edible films. In regards to extending shelf-life, Ponce et al. (2004) showed that 0.05% CBO at 5°C may not increase shelf-life due to sensory issues, but at abuse temperatures it did prolong the shelf-life of organic Swiss chard leaves. CBO also had peroxidase inhibition activity, which supports its potential to extend shelf-life, if used at the appropriate concentrations (Ponce et al., 2004).

Many studies have determined the minimum inhibitory (MIC) and minimum bactericidal concentrations (MBC) of CBO for various bacterial species. When evaluating 10 EOs against strains of *E. coli*, Moreira et al. (2005) found CBO to have the lowest MIC (0.25 ml/100 ml) and MBC (0.3 ml/100 ml), which resulted in reductions of 0.5-3 log CFU/ml for *E. coli* O157:H7 in a time dependent manner. Rhayour et al. (2013) found the MIC of CBO against *E. coli* to be 0.050% (v/v) and the MCB to be 0.1% (v/v). With *B. subtilis*, the MIC was 0.033% (v/v) and the MBC was 0.05% (v/v). Fu et al. (2007) demonstrated that MIC concentrations ranging from 0.062% (v/v) to 0.5% (v/v) had antimicrobial activity against *S. epidermidis*, *E. coli,* and *Candida albicans.* In a review by Burt (2004), the MIC for *E. coli* and *S. aureus* ranged from 0.4-2.5 µl/ml and was >20 µl/ml for *S.* Typhimurium. Bajpai et al. (2012) reported the MIC for *S.* Typhimurium and *S.* Enteritidis being >2 µl/ml and 80-230 µg/ml, respectively.

A majority of the investigations looking into the MOA for CBO are focused on the active component eugenol. Only two studies have directly investigated the MOA using clove EOs or extracts. Wendakoon & Sakaguchi (1995) treated *E. aerogenes* with water and ethanol extracts of clove, exposed their crude extracts to amino acids and concluded that the ethanol extractions of clove inhibited decarboxylases. The authors hypothesized that the phenolic compounds present in the EO reacted with proteins through hydrogen bonding, and ionic and hydrophobic interactions. Rhayour et al. (2003) investigated both CBO and its major phenolic compounds against *E. coli* and *B. subtilis* using SEM and looking at 260 nm leakage. They concluded that the phenolic compounds damage the cell envelope, which ultimately leads to cell death. However, the mechanisms by which they damage the cell envelope were not elucidated.

2.0 Eugenol-Antimicrobial Activity

Eugenol is the active component of CBO and its structure is that of a phenylpropene, which possess a six-carbon aromatic phenol ring with a propene tail consisting of three carbons (Nazzaro et al., 2013). It is a lipophilic molecule like many compounds found in EOs, which may be the source of CBO's antimicrobial activity (Devi et al., 2010; Nazzaro et al., 2013; Di Pasqua et al., 2006). Nascimento et al. (2000) tested the antimicrobial activity of various phytochemicals including benzoic acid, cinnamic acid, eugenol, and farnesol against antibiotic resistant and non-antibiotic resistant *S. aureus, S.* Choleraesuis*, P. aeruginosa, B. subtilis, C. albicans, Proteus spp., Klebsiella pneumoniae, Enterobacter aerogenes*, and *E. coli.* Among the phytochemicals tested, eugenol showed the highest antimicrobial activity. Friedman et al. (2002) demonstrated that eugenol was effective against *C. jejuni, E. coli, L. monocytogenes,* and *S. enterica.*

In regards to the MIC and MBC, Walsh et al. (2003) used 0.1% eugenol to reduce *E. coli* by 4 log CFU/ml and *S. aureus* and *P. aeruginosa* by less than 4 log CFU/ml. The MIC for *E. coli, S. aureus,* and *P. aeruginosa* were determined to be 0.05% (v/v), 0.1% (v/v), and >0.1% (v/v), respectively. A review by Burt (2004) stated that the MIC of eugenol for *E. coli, S.* Typhimurium, and *L. monocytogenes* were 1 µl/ml, 0.5 µl/ml, and >1 µl/ml, respectively. Rhayour et al. (2003) also found the MIC of eugenol against *E. coli* to be 0.05% (v/v) and the MBC to be 0.1% (v/v). For *B. subtilis*, the MIC was 0.033% (v/v) and the MBC was 0.05% (v/v), which were the same as the MIC and MBC of CBO. Devi et al. (2010) reported the MIC of eugenol to be 0.0125% (v/v) and the MBC to be 0.025% (v/v) against *S.* Typhi, which resulted in decreased viability and no detectable survivors, respectively. Furthermore, 5 mM of eugenol was bactericidal to *L. monocytogenes* and 6 mM was bactericidal to *Lactobacillus sakei* (Gill & Holley, 2004). Tippayatum & Chonhenchob (2007) demonstrated the antimicrobial activity of eugenol against *L. monocytogenes, S. aureus, B. cereus* and *E. coli* using MICs of 11 mg/ml, 8 mg/ml, 9 mg/ml, and 8 mg/ml, respectively.

A number of studies have evaluated the MOA of eugenol. Walsh et al. (2003) measured leakage of cell content at 260 nm to conclude that eugenol caused membrane disruption that ultimately lead to cell death but, the mechanism by which it damaged the membrane was not elucidated. Oyedemi et al. (2009) exposed *L. monocytogenes*, *Streptococcus*

pyogenes, *Proteus vulgaris,* and *E. coli* to eugenol and looked at lipid (Van Handel method; Van Handel, 1985) and protein leakage (Bradford method; Bradford, 1976) and concluded that increased leakage of proteins and lipids was simply due to a damaged cell wall and membrane. They hypothesized that the damage to the cell wall was attributed to the hydrophobicity of eugenol and its ability to partition in the lipids of the membrane, which in turn made them more permeable.

Devi et al. (2010) used crystal violet, 260 nm leakage, SDS-PAGE, FT-IR spectroscopy, atomic force microscopy (AFM) and SEM to investigate the effects of eugenol on *S*. Typhi. They also concluded that eugenol decreased the integrity of the membrane causing increased permeability. They hypothesized that the lipophilic/hydrophobic characteristics allow eugenol to leave the aqueous phase and partition into the bacterial membrane causing expansion, increased fluidity and permeability, disruption of proteins, inhibition of respiration, and alteration of ion transport. Gill & Holley (2004) treated *L. monocytogenes* and *L. sakei* with eugenol, measured their intra and extracellular levels of ATP, and found that eugenol appeared to prevent the production of ATP but did not decrease existing ATP levels. The authors hypothesized that the proton gradient of the membrane is not dissipated or the particular enzyme F1F0 ATPase is inhibited. In addition, these authors concluded in another study that eugenol inhibited the motility of *E. coli* and *L. monocytogenes* by potentially altering the PMF and rapidly depleted both intra- and extracellular ATP (Gill & Holley, 2006). This was almost contradictory to their first study, which did not show decreased levels of existing intracellular ATP pools. They concluded that eugenol resulted in nonspecific permeability of membranes (Gill & Holley, 2006). Di Pasqua et al. (2006; 2007) showed changes in the fatty acid profiles of Gram-positive and Gram-negative bacteria exposed to eugenol, but how the changes occurred was not clarified.

3.0 Grape Seed Extract (GSE)-Antimicrobial Activity

Grapes (*Vitis vinifera*) are a common food commodity around the world where they are dried, eaten raw, cooked, or used for wines and juices. Their seeds are considered a byproduct after processing of grape fruit juices and wines, which are then dried and purified to produce an extract. Grape seed extract (GSE) is comprised of various types of compounds, the grape seeds themselves contain 60-79% of the grapes total phenolics, which are known to have antimicrobial properties. These phenolics include hydroquinone, pyrocatechol, caffeic acid, ferulic acid, ϱ-coumaric acid, gallic acid, ellagic acid, and resveratrol. The extracts are also comprised of 74-78% oligomeric proanthocyanidins and less than 6% flavanol monomers (Perumalla & Hettiarachchy, 2011). Jayaprakasha et al. (2003) found procyanidin to be the major component varying from 40-47% based on the extraction method. Corrales et al. (2009) found secondary metabolites from the seeds to be comprised of gallic, protocatechuic, caftaric, ϱ-hydroxybenzoic, and syringic phenolic acids, flavan-3-ols, catechin, epicatechin, trans-polydatin, trans-resveratrol flavonoids, quercetin-3-0-rhamnoside flavonoids, procyanidin B1 and B2, catechin dimers, epicatechin gallate, and trimer proanthocyanidins.

In addition, GSE is known to possess a variety of bioflavonoids, which are known to have antioxidant activity by acting as free radical scavengers (Al-Habib et al., 2010). Doses commonly used in pharmacological applications range from 150-300 mg/day and much lower concentrations of 0.01-1% are used in food applications (Perumalla & Hettiarachchy, 2011).

Corrales et al. (2009) evaluated the efficacy of GSE at inhibiting *L. monocytogenes*, *S*. Typhimurium, *S. aureus*, *E. coli, Enterococcus faecium, E. faecalis*, and *Brochothrix thermosphacta.* The authors found that GSE inhibited the Gram-positive foodborne pathogens but not Gram-negatives. Jayaprakasha et al. (2003) tested GSE against *B. cereus, B. coagulans, B. subtilis, S. aureus, E. coli*, and *P. aeruginosa.* Concentrations of 850-1000 ppm inhibited the Gram positives and concentrations of 1250-1500 ppm inhibited the Gram negatives. Friedman et al. (2013) demonstrated that GSE at a concentration of 0.015% (w/v) killed 50% of *S. aureus* population.

Furthermore, GSE has also been shown to be effective against antibiotic resistant bacteria. For instance, Al-Habib et al. (2010) tested GSE against 43 strains of MRSA by gel diffusion and found that all strains were sensitive to the extract with complete inhibition observed at a concentration of 3 mg/ml. Brown et al. (2009) found that grape skin was more effective but GSE did inhibit *H. pylori in vitro* and reduced or inhibited the ability of *H. pylori* to bind to or damage atypical glandular (AGS) cells. GSE has shown antimicrobial activity in various foods such as frankfurters, poultry products, raw meats, fish, and tomatoes.

Ahn et al. (2004) showed that GSE at 1% w/w inhibited *E. coli* O157:H7 and *S*. Typhimurium in cooked ground beef (Perumalla & Hettiarachchy, 2011). In addition, Corrales et al. (2009) incorporated GSE into pea starch films and

tested their efficacy at reducing *B. thermosphacta* populations on pork loins where it reduced bacterial populations by 1.3 log CFU/g after four days at 4°C.

Limited studies have specifically investigated the MOA of GSE; however, hypotheses about the mechanism are made while discussing the observed antimicrobial activity. For instance, Al-Habib et al. (2010) used SEM to conclude that the GSE most likely targets the cell wall or membrane. Corrales et al. (2009) hypothesized that the polyphenols present in GSE are able to penetrate the membrane and react with intracellular proteins. Specifically, they hypothesized that caffeic acid, which possesses a propanol side chain, may facilitate transport across the cell membrane and that tannins may inhibit enzymes. Furthermore, Tesaki et al. (1999) suggested that the three hydroxyl groups and substituents of the benzene rings of gallic acid found in GSE were responsible for the activity observed against *S. aureus* (Jayaprakasha et al., 2003). Perumalla & Hettiarachchy (2011) hypothesized that the MOA may be due to metal chelating properties, reduction of hydrogen peroxide formation, effects on cell signaling pathways, and gene expression. However, no work has been done that directly focuses on these mechanisms for GSE.

4.0 Olive Extract-Antimicrobial Activity

Olives *(Oleo europaea)* also contain components that are considered agricultural byproducts such as their leaves, pulp, or juice, which can be dried and purified to become extracts. The extracts from these byproducts can consist of hydroxytyrosol, tyrosol, caffeic acid, ϱ-coumaric acid, vanillic acid, vanillin, oleuropein, luteolin, diosmetin, rutin, verbascoside, luteolin-7-glucoside, apigenin-7-glucoside, and diosmetin-7-glucoside (Lee & Lee, 2010; Pereira et al., 2007). Compounds found in olive extracts such as 4-hydroxytyrosol are known to have antioxidant, anticancer, anti-cholesterol, anti-aging, antidiabetic, and antimicrobial properties as well as protect against bone loss, heart disease, oxidative injury of kidney cells, and suppress oxidative stress (Friedman et al., 2011).

Most antimicrobial studies using olive extracts are focused on evaluating the active component oleuropein, the compound responsible for the bitter property of olives, or hydroxytyrosol, which is derived from oleuropein by enzymatic hydrolysis (Bisignano et al., 1999). However, a few have evaluated the efficacy of whole extracts. Sudjana et al. (2009) screened the antimicrobial activity of olive leaf extract against 122 organisms and found it was the most effective against *H. pylori, S. aureus* including MRSA strains, and *C. jejuni* with low MICs ranging from 0.31-0.78% (v/v). The authors concluded that olive leaf extract is not broad spectrum and may only be applicable against these organisms. However, Friedman et al. (2013) evaluated olive pomace and olive juice powder at concentrations ranging from 0.125-4% and found broad-spectrum activity against *E. coli* O157:H7, *S. enterica, L. monocytogenes,* and *S. aureus.* They also tested the active components and found that hydroxytyrosol was effective against all four pathogens, whereas oleuropein was only effective against *S. aureus* and *S. enterica.* The olive juice powder was the most effective against *S. aureus* and *L. monocytogenes* at the same concentrations as the pure hydroxytyrosol, suggesting that there may be other phenolics present in the olive juice powder that could contribute to the enhanced antibacterial activity.

Pereira et al. (2007) also reported broad-spectrum antimicrobial activity and found olive leaf extracts to be effective against *P. aeruginosa, K. pneumoniae* as well as fungi including *C. albicans* and *C. neoformans*. In addition, these authors stated that the active components hydroxytyrosol and oleuropein are effective against intestinal and respiratory tract pathogens such as *Haemophilus influenzae, Moraxella catarrhalis, S.* Typhi*, Vibrio parahaemolyticus, V. cholera,* and *V. alginolyticus*.

Furthermore, using disc diffusion assay, Lee & Lee (2010) demonstrated that oleuropein at 800 μg inhibited *S.* Enteritidis with a zone of inhibition of 23.5 mm diameter, and caffeic acid moderately inhibited growth of *B. cereus, E. coli,* and *S.* Enteritidis with zones of inhibition of 9.8-10.4 mm diameter, but no effect was observed on *S. aureus*, which was contradictory to other studies. In addition, mixtures of the phenolic compounds such as oleuropein, rutin, vanillin, and caffeic acid showed greater antimicrobial activity than their individual use, suggesting synergistic properties of the olive leaf extract.

Studies have implicated the efficacy of olive extracts and the active components against bacterial toxin production. Friedman et al. (2011) evaluated the antimicrobial activity of pure 4-hydroxytyrosol at concentrations of 0.022-0.71 mg/ml and a commercial olive powder (Hidrox-12) made from freeze dried olive juice at concentrations of 0.04-1.29 mg/ml against *S. aureus* and its toxin production. Growth was completely inhibited and Toxin A was inactivated with the pure 4- hydroxytyrosol at concentrations that were not found to be toxic to cultured murine spleen cells; however, the inhibitory concentrations of the Hidrox-12 product were found to be toxic to spleen cells. In

addition, Tassou & Nychas (1994) found that oleuropein (0.6%) and other phenolics extracted from olives, inhibited enterotoxin B production by *S. aureus* in broth medium as well as in a milk food model. As stated previously, the lower pH of the milk enhanced the effectiveness of the extract. This was similar to the results of Tranter et al. (1993) who showed that oleuropein at concentrations between 0.4% and 0.6% completely inhibited growth of *S. aureus,* and concentrations greater than 0.2% inhibited the production of enterotoxin B.

Limited research has been done on the exact MOA of olive extracts and their active components. As mentioned previously, it has been hypothesized that the MOA of these compounds is attributed to the phenolic compounds found in the extracts, which generally target the cell membrane and may interact with proteins (Burt, 2004; Bisignano et al., 1999). Juven et al. (1972) treated *Lactobacillus plantarum* with oleuropein, which resulted in leakage of glutamate, potassium, and inorganic phosphate as well as decreased levels of intracellular ATP. Zanichelli et al (2004) made use of oxidative stress assays and enzymatic determination methods to study the MOA of oleuropein. They suggested that the MOA of oleuropein is driven by interactions with hydrogen peroxide and involves redox mechanisms to inhibit bacterial growth. In laboratory media containing tryptone, the authors hypothesized that oxidation of tryptone by *S. aureus* resulted in the production of peroxides that oleuropein could then break down via redox reactions and contribute to the formation of more hydrogen peroxide. However, this MOA is highly debatable due to the experimental approach where hydrogen peroxide alone did not kill but when paired with oleuropein, it inhibited *S. aureus*, indicating a synergistic effect between the two compounds (Zanichelli et al., 2004).

5.0 Combination Treatments

The organoleptic impact of applying EOs to food is a notable concern by many. In an attempt to minimize these effects, researchers have investigated the potential use of the major and minor components of EOs and extracts individually. However, it appears that the minor components do contribute to the antimicrobial activity where the major components or their mixtures were not as effective as the EO or extract as a whole (Burt, 2004). It has been suggested that combining EOs with plant extracts may allow a reduction in the concentrations of the EO needed to achieve adequate antimicrobial activity while minimizing the impact on the organoleptic profile of treated foods (Gutierrez et al., 2008; Lambert et al., 2001). Combinations may be useful not just for sensory attributes, but also when the EO or extract is not substantially effective on its own, by allowing for multiple targets and mechanisms of action from multiple compounds (Perumalla & Hettiarachchy, 2011; Wagner & Ulrich-Merzenich, 2009).

Additive, synergistic, and antagonistic activities have been studied by a number of researchers using various combinations of EOs and their active components (Burt, 2004; Gutierrez et al., 2008). Delaquis et al. (2002) combined extracts of cilantro, dill, eucalyptus, and coriander EOs and demonstrated additive, synergistic, or antagonistic activity when mixed in varying combinations. In addition, Lv et al. (2011) used combinations consisting of oregano-basil, oregano-bergamot, basil-bergamot, and oregano-perilla EOs against *S. aureus, B. subtilis, E. coli,* and *Saccharomyces cerevisiae*. All combinations were synergistic against *S. aureus*, the oregano with basil or bergamot oils had additive effects on *B. subtilis*, and the oregano with basil or perilla oils had additive effects on *E. coli* and yeast. The authors also concluded that EO combinations can inhibit the growth of bacteria at lower concentrations than were needed when the EOs were used individually.

In regards to combinations of active components, Moleyar & Narasimham (1992) showed that a combination of cinnamaldehyde and eugenol inhibited growth of *Bacillus, Staphylococcus, Micrococcus,* and *Enterobacter* species for over 30 days, whereas their individual applications at the same concentrations did not inhibit growth (Burt, 2004). Al-Bayati (2008) also showed additive effects with combinations of phytochemicals against *P. aeruginosa*, which was found to be resistant to all of the EOs and methanol extracts when they were used individually.

Another strategy prevalent in the literature is the use of EOs, extracts, or their active components with antibiotics or other common antimicrobials as a multiple hurdle approach. Hemaiswarya et al. (2008) describe how secondary metabolites from plants can modify mechanisms for multi drug resistance through synergistic activity with synthetic drugs. Clove extract showed synergistic activity with 11 different antibiotics that target protein, folic acid, nucleic acid, and cell wall synthesis at 1/4 MIC against various strains of *S. aureus,* while others such as ginger and garlic extracts only showed synergism with two or three of the drugs, respectively. Lemongrass and guava extracts also exhibited high synergistic activity with the antibiotics (Betoni et al., 2006). Nascimento et al. (2000) showed that extracts of clove, pomegranate, thyme, and jambolan were effective against *P. aeruginosa* and other bacterial species but more

interestingly, synergistic activity was also achieved using lower concentrations of the EOs and extracts paired with antibiotics that were usually ineffective against this pathogen.

Furthermore, Kim et al. (2012a) demonstrated that rice hull smoke extract had synergistic antimicrobial activity against. *S*. Typhimurium *in vitro* and *in vivo* when combined with vancomycin. In regards to combinations with commonly used antimicrobials, Theivendran et al. (2006) found that 1% GSE with 10,000 IU/ml of nisin had a 9 log CFU/ml reduction of *L. monocytogenes* in PBS. In addition, when the 1% GSE and 10,000 IU/ml of nisin was incorporated into soy protein films and applied to turkey frankfurters, they observed more than a 2 log CFU/g reduction.

Fyfe et al. (1998) evaluated combinations of fennel, anise, basil oils and benzoic acid or methyl-paraben against *S*. Enteritidis and *L. monocytogenes*. Their data showed that *S*. Enteritidis was sensitive to combinations of anise, fennel, or basil EOs with methyl-paraben, which resulted in bacterial populations under 10 CFU/ml, indicating a 3.0 log reduction after one hour of incubation at 37°C. *L. monocytogenes* was less sensitive to the combinations but still showed 4-8 log CFU/ml reductions with the combinations of all the oils and methyl-paraben with incubation times of 8-48 hours at 37°C. In nearly all of these cases, the antimicrobial activity observed with the combination treatments was greater than the activity seen when each of these compounds were used individually at the same concentrations.

6.0 Plant Antimicrobial Edible Films

For preparation of antimicrobial edible films, various plant antimicrobials are incorporated into the processed pulps of fruits, vegetables and flowers which are then cast into thin films, dried and cut into the required shapes and sizes. These edible films containing plant antimicrobials can be incorporated into sealed salad bags or wrapped onto pieces of meat.

Ravishankar et al. (2009) evaluated the efficacy of apple films containing cinnamaldehyde or carvacrol against foodborne pathogens inoculated on ham and chicken breast. *S. enterica* or *E. coli* O157:H7 cultures were surface inoculated on chicken breasts and *L. monocytogenes* on ham. The meats were wrapped with the edible films containing various concentrations of the antimicrobials and stored at room and refrigeration temperatures. In general, carvacrol exhibited stronger activity against all the microorganisms on both meats in comparison to cinnamaldehyde (Ravishankar et al., 2009).

Surface contamination is one of the most common ways by which foodborne pathogens find their way into deep tissues. Ravishankar et al. (2012) have assessed the efficacy of edible films on ready to eat meats such as ham and bologna. The films were made of apple, carrot and hibiscus pulps with carvacrol and cinnamaldehyde added as antimicrobials. The meat products were inoculated with *L. monocytogenes* and wrapped with these edible films. When bacterial survivors were enumerated, considerable reductions were seen with films containing carvacrol. Compared to the control films which had no antimicrobials, films with 3% carvacrol induced 1-3, 2-3, and 2-3 log CFU/g reductions on ham and bologna at day 0, 3, and 7, respectively. The pectin based apple films were found to be more effective as compared to carrot and hibiscus films (Ravishankar et al., 2012). Furthermore, films containing carvacrol were found to be more effective than those containing cinnamaldehyde. In general the edible films were found to be more effective on ham in comparison to bologna, indicating that the composition of the meat, including nutritive and non-nutritive compounds, could have an impact on the efficacy of the antimicrobials (Ravishankar et al., 2012).

Mild et al. (2011) studied the effect of edible apple films containing cinnamaldehyde and carvacrol on chicken breasts that were inoculated with *Campylobacter jejuni*. Both carvacrol and cinnamaldehyde showed bactericidal activity against *C. jejuni* when used in edible apple films on chicken. Cinnamaldehyde had higher activity against *C. jejuni* compared to carvacrol (Mild et al., 2011).

Zhu et al. (2014) evaluated edible films made from carrot, apple and hibiscus infused with carvacrol and cinnamaldehyde in salad bags and found them to be effective against *Salmonella* inoculated onto lettuce and spinach. The inoculated greens were placed in salad bags and sealed after edible films were added to them. At the end of the week, Hibiscus films with 3% cinnamaldehyde reduced the bacterial population to below detectable limits (Zhu et al., 2014). Apple and carrot films with the same concentration of cinnamaldehyde showed a reduction of 0.7-2.7 and 0.6-1.9 log CFU/g, respectively. Among the films containing carvacrol, all 3 types of edible films with 3% carvacrol reduced

the bacterial population to below detectable limits by day 0 (Zhu et al., 2014). This indicates that carvacrol exhibited a stronger antimicrobial action against *Salmonella* as compared to cinnamaldehyde.

Certain foodborne pathogens associated with specific foods indicate that these microorganisms are resistant to numerous processing techniques. The use of antimicrobials that exhibit residual activity during storage indicate that these compounds could be the newer alternatives to antibiotics and other potentially harmful chemical substances used in food production. When incorporated into films made from natural plant based pulps, the moisture from the foods allows for the diffusion of the antimicrobials into the foods, allowing for the reduction in population of foodborne microorganisms.

Plant Antimicrobial Treatments on Produce

It has been noted that produce may be a food type more suitable for the application of EOs and extracts when compared to meats or dairy products. This is because produce has minimal limiting factors that could influence the antimicrobial activity of plant compounds, such as the high fat or protein content, that may be present in other types of foods such as meats. In fact, the antimicrobial activity of EOs and extracts has been shown to benefit from the low pH and storage temperatures usually associated with fresh produce (Burt, 2004). A number of studies have evaluated the efficacy of EOs and extracts against a broad range of foodborne pathogens on/in produce and meat products in addition to *in vitro* assays. For instance, Fisher & Phillips (2006) demonstrated the effectiveness of lemon, orange, and bergamot EOs and their active components against *C. jejuni, E. coli* O157:H7*, L. monocytogenes, B. cereus,* and *S. aureus* both *in vitro* and in foods such as cabbage leaves. Results showed that bergamot, citral, and linalool were the most effective *in vitro* and were then tested on cabbage leaves. Citral and linalool vapors reduced *L. monocytogenes, B. cereus,* and *S. aureus* populations by 6 log CFU/g on cabbage leaves after 8-10 hours of exposure.

Kim et al. (2011) treated lettuce leaves with 1%, 5%, and 10% clove extracts for 0, 1, 3, 4, and 10 min to test their efficacy at reducing *S*. Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* populations. Following the 10 minute treatment at 5% and 10% concentrations, *S*. Typhimurium and *E. coli* O157:H7 populations were reduced by about 3 to 4 log CFU/g. However, the higher concentrations only reduced *L. monocytogenes* by 1 log or less.

Gündüz et al. (2009) treated shredded iceberg lettuce and whole tomatoes contaminated with *S*. Typhimurium using concentrations of myrtle oil ranging from 500-1000 ppm for exposure times ranging from 5-20 min. Their results showed a maximum reduction of 1.66 and 1.89 log CFU/g for iceberg lettuce and tomatoes, respectively. The authors stated that the activity was treatment time dependent. The same authors also tested the efficacy of oregano oil against *S*. Typhimurium on iceberg and romaine lettuce. Reductions did not exceed 1.92 log CFU/g for all washing times (5, 10, 15, and 20 min) and concentrations (25, 40, and 75 ppm) evaluated.

Moore et al. (2011) evaluated the efficacy of apple, hibiscus, and olive extracts in comparison to hydrogen peroxide against antibiotic resistant *S*. Newport on four organic leafy greens; iceberg lettuce, romaine lettuce, baby spinach and mature spinach. Concentrations of 1%, 3%, and 5% (w/v) were evaluated for the apple and olive extracts and 10%, 20%, and 30% (w/v) for the hibiscus extract. The organic leafy greens were treated for 2 min, stored at 4°C, and samples were taken on days 0, 1 and 3. Reductions on all four leafy greens treated with olive extract ranged between 2-3 log CFU/g by day 3, apple extract reduced *Salmonella* population between 1-2 log CFU/g by day 3, and hibiscus extract had an overall reduction of 1 log CFU/g (Moore et al., 2011). The observed activity appeared to be concentration and storage time dependent. These results suggest that EOs or extracts may serve as a suitable alternative for sanitizing fresh produce.

Plant Antimicrobial Treatments for Meat Products

Chen et al. (2013) studied the effectiveness of antimicrobials such as cinnamon and oregano oils and olive and apple extracts against multidrug resistant *S*. Typhimurium DT104 during cooking (70°C for 5 min) and subsequent cold storage of inoculated ground pork at 4°C for 7 days. The antimicrobials were found to be stable during both heating and cold storage, since the populations of *Salmonella* recovered from both treated and control pork samples were similar (Chen et al., 2013). The antimicrobial activity of cinnamon oil and olive extract in heated ground pork during storage at 4°C up to 7 days was evaluated, and 1.3 and 3 log CFU/g reductions were obtained with 1% cinnamon oil and 5% olive extract, respectively (Chen et al., 2013). In this study, Chen et al. (2013) also found that the minimum concentration of the antimicrobials required to cause >1 log reduction in *S*. Typhimurium was 0.8% for cinnamon oil and 4% for olive extract.

Meat products need to be heated to high temperatures that ensure the killing of all pathogens present in the raw material. However, during this process, due to the biochemical composition of meat, carcinogenic compounds called heterocyclic amines (HCAs) are formed (Knize et al. 2005). HCAs are formed in muscle tissues that are rich in proteins. The reaction is a complex third order condensation reaction involving compounds commonly found in animal based, protein-rich foods such as creatinine, tryptophan (or another amino acid) and glucose or other sugars as reactants (Cross and Sinha, 2004).

Studies have shown that the incorporation of plant antimicrobials into the beef patties is efficient in killing the pathogens that may be present, thus reducing the need for such high heat to be used (Friedman et al., 2009; Rounds et al., 2012; Rounds et al., 2013). This approach wherein the use of heat is reduced or minimized because of the incorporation of other antimicrobial substances, is called the multiple hurdle approach. Friedman et al. (2009) showed that carvacrol mixed into beef patties inoculated with *E. coli* O157:H7 and grilled showed a simultaneous reduction in the *E. coli* O157:H7 population (2.5-5 l logs) and the levels of 3 major heterocyclic amines (58-78% reduction).

Rounds et al. (2012) evaluated essential oils, common spice powders and plant extracts for their effectiveness against *E. coli* O157:H7 and heterocyclic amines in grilled meat patties. *E. coli* O157:H7 population was reduced by 1.6 log CFU/g-below detection limits and heterocyclic amines were reduced by 24.2-94.3%. Olive extract and lemongrass oil reduced *E. coli* O157:H7 population to below detection limits, while onion powder, olive extract and apple extract cause 94.3, 84.3, and 82.1% reductions in one of the heterocyclic amines (PhIP), respectively (Rounds et al., 2012). Olive and apple extracts and clove bud essential oil showed both antimicrobial and anti-oxidative activity reducing both *E. coli* O157:H7 and heterocyclic amines (Rounds et al., 2012). Rounds et al (2013) evaluated the concentration dependent activity of apple and olive extracts, onion powder and clove bud oil against *E. coli* O157:H7 and heterocyclic amines in grilled hamburger patties. *E. coli* O157:H7 populations were reduced by 1 log-below detection limits, MeIQx was reduced by 47-50.9% and PhIP was reduced by 50.6-80.7% by the addition of optimal amounts of the selected plant compounds (Rounds et al., 2013).

Conclusion

When investigating the antimicrobial activity of EOs or extracts in foods, it has been found that higher concentrations are needed when compared to the *in vitro* studies (Gutierrez et al., 2008; Bajpai et al., 2012). Properties of the food matrix that may affect the activity of EO and extracts include; the pH, with greater activity being observed at a lower pH due to increased hydrophobicity of the EO, the temperature at which the food is stored, the amount of oxygen present, as well as the water, protein, and fat content. High fat content appears to decrease the antimicrobial activity by potentially providing protection for the bacteria (Burt, 2004; Gutierrez et al., 2008). Many have proposed the use of EOs and plant extracts as natural alternatives to chemicals commonly used in the food industry to prevent or reduce contamination by foodborne pathogens (Fisher & Phillips, 2006 Ravishankar et al., 2010, Moore et al., 2011, Rao, 2017). The use of EOs and extracts as natural antimicrobials or preservatives appeals to the "green consumerism" that has emerged over the past couple of decades, while providing a means to protect the public from foodborne illness. Wilkins et al. (1989) stated that over 1,340 plants are potential sources of compounds with antimicrobial activity, but only a handful have been studied (Elgayyar et al., 2001). Overall, many plant compounds have demonstrated their efficacy against a number of foodborne pathogens *in vitro* and in model food systems, and hence, have potential to be

applied in foods to improve their microbiological safety. Further research will help in commercial applications of these compounds in the food industry.

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