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Antimicrobial efficacy of plant phenolic compounds against *Salmonella* and *Escherichia Coli*

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ABSTRACT

The present study evaluated the antimicrobial efficacy of natural phenolic compounds (PC) extracted from vegetables, fruits, herbs and spices; to inhibit the growth of Gram-negative foodborne bacteria which is defined as the minimum inhibitory concentration (MIC).

Strains of *Escherichia coli* and *Salmonella* species were treated with natural PCs including; chlorogenic acid, curcumin, (−) epicatechin, eugenol, myricetin, quercetin, rutin, thymol, thymoquinone, and xanthohumol. Concentrations of 5, 10, 15, and 20 ppm of each compound were evaluated by a broth micro-dilution method and the MICs were determined by using optical density after 24 and 60 h of incubation. Structural alterations in treated bacteria were observed *via* scanning electron microscopy.

For *E. coli*, thymoquinone showed the highest inhibition, followed by rutin, (−) epicatechin and myricetin (MIC < 20 ppm for all), while *Salmonella* was most sensitive to (−) epicatechin (MIC < 15 ppm), followed by thymoquinone, rutin and myricetin (MIC < 20 ppm for all) following 60 h of incubation. The results demonstrated that the PCs have varying antimicrobial activities against foodborne pathogens following 24 and 60 h incubation periods. Natural sources of PCs contain major antibacterial components and have great potential to be used as natural antimicrobials and food preservatives, during long term storage.

This study highlighted the antimicrobial efficacy of some novel PCs which may replace chemical antimicrobials and preservatives in food or pharmaceutical industry to partially or completely inhibit the growth of bacteria.

1. Introduction

Spices and aromatic vegetable materials have long been used in food not only for their flavor and aromatic qualities and appetizing effects but also for their preservative and medicinal properties. Since ancient times, they have been used for preventing food spoilage and deterioration and extending the shelf life of foods (Gyawali & Ibrahim, 2014; Shan, Cai, Brooks, & Corke, 2007b). It has been extensively reported that the essential oils and secondary plant metabolites have shown antimicrobial activities against foodborne pathogens (Reichling, Schnitzler, Suschke, & Saller, 2009; Smith-Palmer, Stewart, & Fyfe, 1998). In addition, they have received great attention in recent decades due to their presumed role in the prevention of foodborne diseases, cancer, chronic and cardiovascular diseases, and in the slowdown of the aging process (Lee, Cesario, Wang,

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Shanbrom, & Thrupp, 2003; Liu, 2003; Nazzaro et al., 2009; Lou, Wang, Zhu, Ma, & Wang, 2011) indicating that, they have diverse beneficial biological functions including antimicrobial and antioxidant activities (Korukluoglu, Sahan, Yigit, Ozer, & Gucer, 2010; Perumalla & Hettiarachchy, 2011).

Phenolic compounds (PC) are one of the most diverse groups of secondary metabolites found in a wide variety of fruits, vegetables, nuts, seeds, stems and flowers as well as tea, wine, propolis and honey (Moreno, Scheyer, Romano, & Vojnov, 2006). The exploration of natural antimicrobials for food preservation receives increased attention due to consumer awareness of natural food products and a growing concern of microbial resistance towards conventional food processing and preservation methods. The use of PCs as antimicrobial agents could potentially provide additional benefits, including dual-function effects of both preservation and delivery of health benefits (Cueva et al., 2010). By gaining the fundamental knowledge on the antimicrobial effects of plant derived PCs on pathogenic microorganisms, it is possible to search new strategies to combine the synergic antimicrobial effects of PCs with their natural biological properties. The results may permit the formulation of new products to be used as food preservatives or to be included in the human diet. Thus, the food industry is interested in developing natural components for the total or partial replacement of synthetic antimicrobials (Santas, Almajano, & Carbo, 2010).

Mechanisms of action in the bacterial cell of bioactive plant compounds such as degradation of the cell wall (Nychas and Tassou, 1999), damage to cytoplasmic membrane and membrane proteins (Lambert, Skandamis, Coote, & Nychas, 2001), leakage of contents out of the cell, coagulation of cytoplasm, and depletion of the proton motive force (Burt, 2004; Gyawali & Ibrahim, 2014) have been reported. In general, variations in antimicrobial activities against bacteria may reflect differences in cell surface structures between Gram-negative and Gram-positive species; Gram-positive being more susceptible to the action of phenolic acids than Gram-negative bacteria (Cueva et al., 2010). Also, the number, type and position of substituents in the benzene ring of the phenolic acids and the saturated side-chain length influence the antimicrobial potential of the phenolic acids against different microorganisms (Gill & Holley, 2006).

Inhibitory effects of several natural bioactive compounds from berries and grapes (Puupponen-Pimia et al., 2001; Zanoli & Zavatti, 2008) herbs and spices (Albayrak, Aksoy, Sagdic, & Albayrak, 2012; Shan et al., 2007b; Shan, Cai, Brooks, & Corke, 2007a) and other plant extracts (Pereira et al., 2007b; Raybaudi-Massilia, Mosqueda-Melgar, Soliva-Fortuny, & Martin-Belloso, 2009; Santas et al., 2010) were tested against food-relevant bacteria including: *E. coli*, *Salmonella*, *Listeria*, and *Staphylococcus*. Although the antimicrobial activity of some PCs including: thymol (Lambert et al., 2001; Santas et al., 2010), eugenol (Phanthong, Lomarat, Chomnawangb, & Bunyapraphatsaraa, 2013), carvacrol (Lambert et al., 2001; Santas et al., 2010), quercetin (Yao et al., 2011) and myricetin (Puupponen-Pimia et al., 2001) has been previously reported, the response after the long term exposure was not reported and there are still many unexplored sources. Moreover, thymoquinone and xanthohumol have not been included in antimicrobial studies and antimicrobial activity of chlorogenic acid, curcumin, (−) epicatechin, eugenol, myricetin and rutin have not been reported broadly on pathogenic *Salmonella* and *E. coli*.

Objectives of this study are: (1) to evaluate the antimicrobial activity of selected natural PCs extracted from herbs, spices, vegetables, and fruits against Gram-negative foodborne pathogens: *E. coli*, *E. coli* O157:H7, *Salmonella paratyphi*, *Salmonella cholerasuis subsps. cholerasuis* and *Salmonella Enteritidis*, (2) to determine the MIC of the natural PCs and (3) to observe their prolonged antimicrobial activities over extended incubation of 60 h to simulate the long term storage by extending the exposure time of pathogens to PCs.

2. Materials and methods

2.1. Preparation of phenolic compounds

The natural PCs used in this study are: chlorogenic acid, curcumin, (−) epicatechin, eugenol, myricetin, quercetin, rutin, thymol, thymoquinone, and xanthohumol (Sigma-Aldrich, St Louis, MO, USA). Each compound was prepared in ethanol, (95%) (Decon Laboratories, King of Prussia, PA, USA) except for thymoquinone, which was prepared with dimethyl sulfoxide 99.9% (DMSO) (Fisher Scientific, Fair Lawn, NJ, USA). The final phenolic solution was adjusted to approximately pH 5.00 using HCl (15%) to ensure that the pH would not affect the bacterial growth. All solutions were filter sterilized using 0.2 μm filters (Millipore Corporation, Billerica, MA, USA) and stored at 4 °C in sterilized sealed glass containers until needed. All measurements were done at ambient temperature.

2.2. Bacterial strains, culture conditions and preparation of inoculums

Foodborne pathogens including: *E. coli*, F. T. Jones (FTJ), *E. coli* O157:H7, ATCC 43895 and *E. coli* O157:H7, ATCC 35150, *S. paratyphi*, UK Micro 29A (UKM 29A), *S. cholerasuis subsp. cholerasuis*, ATCC 10708 and *S. Enteritidis*, UK (−) H_2S were supplied from the American Type Culture Collection and the University of Kentucky. Bacteria were grown and maintained on slants of brain–heart infusion (BHI) agar and stored at 4 °C until needed. Prior to each test, at least three consecutive transfers of the cultures were inoculated in BHI broth and they were incubated overnight at 37 °C. Then, the inoculums were standardized according to a MacFarland 0.5 turbidity standard (10^8 CFU ml^{-1}) by diluting the sample (Taguri, Tanaka, & Kouno, 2004). Culture growth turbidity, which is indicated by the optical density (OD), was adjusted for each bacterium at a wavelength of 660 nm (OD_{660}) by using the spectrophotometer (BioTek Synergy 4, Winooski, VT, USA) to the final concentrations of approximately 10^7–10^8 CFU ml^{-1}. Cell counts were confirmed by using a spiral plating method with Plate Count Agar (PCA) and the Eddy Jet spiral plater (Neutec Group Inc., Farmingdale, NY, USA). The counts were determined by the Flash and Go plate reader (Neutec Group Inc., Farmingdale). All microbiological media and supplements used in the study were supplied from Difco Laboratories (Sparks, MD, USA).