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Review

Overview of antibacterial, antitoxin, antiviral, and antifungal activities of tea flavonoids and teas

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Tea leaves produce organic compounds that may be involved in the defense of the plants against invading pathogens including insects, bacteria, fungi, and viruses. These metabolites include polyphenolic compounds, the six so-called catechins, and the methyl-xanthine alkaloids caffeine, theobromine, and theophylline. Postharvest inactivation of phenol oxidases in green tea leaves prevents oxidation of the catechins, whereas postharvest enzyme-catalyzed oxidation (fermentation) of catechins in tea leaves results in the formation of four theaflavins as well as polymeric thearubigins. These substances impart the black color to black teas. Black and partly fermented oolong teas contain both classes of phenolic compounds. A need exists to develop a better understanding of the roles of polyphenolic tea compounds in food and medical microbiology. This overview surveys and interprets our present knowledge of activities of tea flavonoids and teas against foodborne and other pathogenic bacteria, virulent protein toxins produced by some of the bacteria, virulent bacteriophages, pathogenic viruses and fungi. Also covered are synergistic, mechanistic, and bioavailability aspects of the antimicrobial effects. Further research is suggested for each of these categories. The herein described findings are not only of fundamental interest, but also have practical implications for nutrition, food safety, and animal and human health.

Keywords: Antibacterial effects / Antitoxin effects / Antiviral effects / Tea flavonoids / Teas

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1 Introduction

Food growers and processors, food safety researchers, regulatory agencies, microbiologists, virologists, epidemiologists, nutritionists, pharmacists, physicians, veterinarians, and, of course, the general public have been increasingly concerned with the growing number of foodborne illness outbreaks caused by some pathogens. These manifestations are exacerbated by increasing antibiotic resistance of some pathogens associated with foodborne illness and by overconsumption of medicinal antibiotics. Therefore, there has been increasing interest in developing novel types of effective plant-derived antimicrobial compounds, including those present in tea leaves (*Camellia sinensis*).

Pathogenic strains of *Bacillus cereus*, *Campylobacter jejuni*, *Clostridium perfringens*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enterica*, and *Staphylococcus aureus* are linked to foodborne illnesses. Safe plant-derived products (botanicals, phytochemicals) are a source of compounds that may provide useful interventions to reduce pathogens in foods. In an effort to define the chemical basis for bactericidal effects of natural compounds, we determined the antimicrobial effects of about 200 plant essential oils and their active components, phenolic compounds, tea catechins and theaflavins, and tea infusion against pathogenic bacteria [1–3]. In related studies, we showed also that in the selected compounds active against nonresistant bacteria were also active against antibiotic-resistant bacteria [4–6] as well as in apple juices [7], tomato and other vegetable juices [8], wines [9], and in ground beef and turkey [10–12]. These studies offer insights into structural features that govern bactericidal activities as well as providing candidates for use in formulations to reduce pathogens in foods.

Commercial teas are usually classified into three major categories: unfermented containing catechins; fully fer-

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Abbreviations: ECG, epicatechin-3-gallate; **EGCG,** (–)-epigallocatechin-3-gallate; **HIV,** human immunodeficiency virus; **HSV,** herpes simplex virus; **LF,** lethal factor; **MIC,** minimum inhibitory concentration

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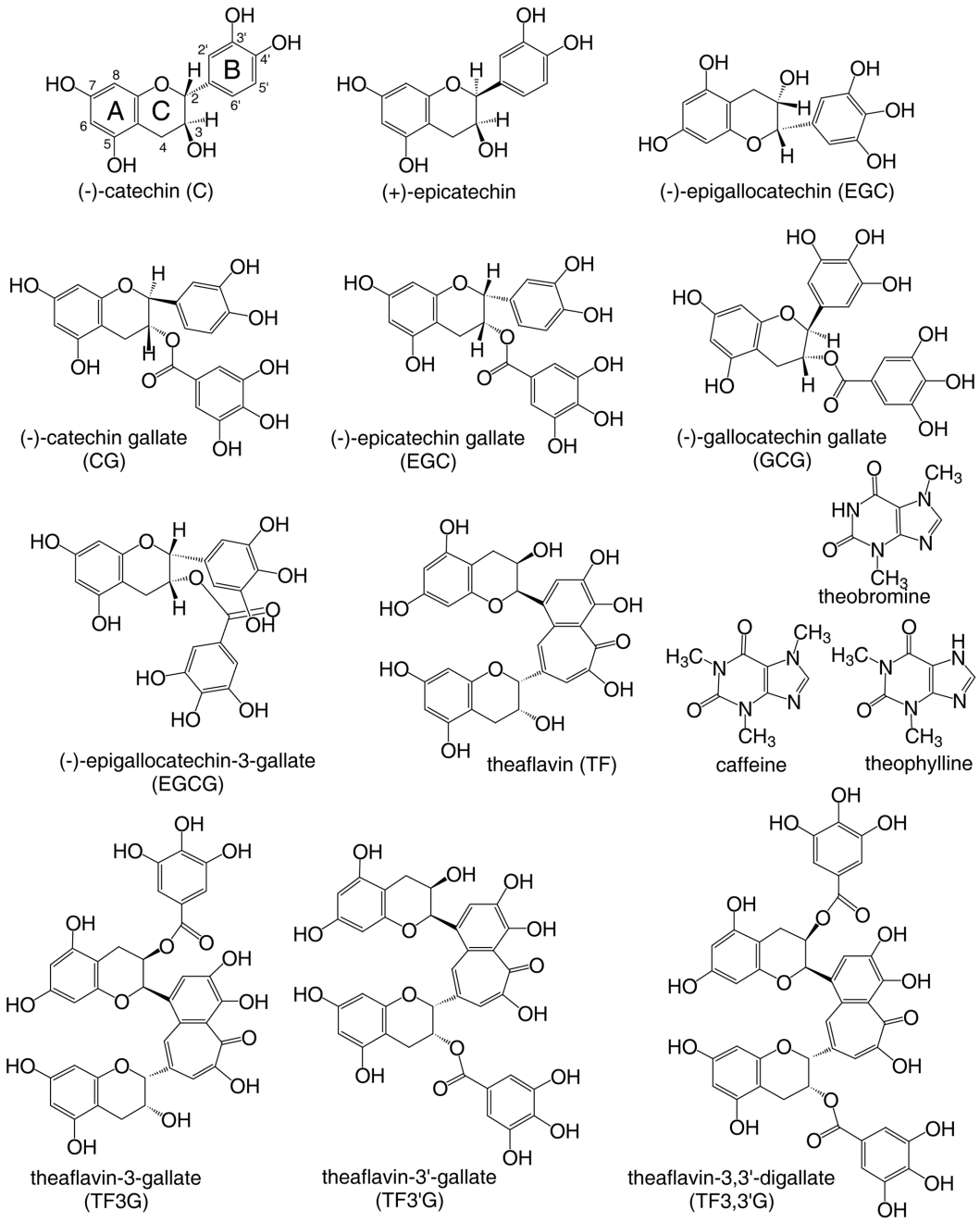

Figure 1. Structures of tea flavonoids (six green tea catechins, four black tea theaflavins) and alkaloids (caffeine, theobromine, theophylline). The indicated abbreviations are used in the text.

mented black tea containing catechins, theaflavins, and polymeric thearubigins; and semifermented usually black oolong, containing both catechins and theaflavins. A catechin can exist as one of two geometrical isomers depending on the stereochemical configuration of the 3′,4′-dihydroxyphenyl and hydroxyl groups at the 2- and 3-positions of the C-ring: *trans*-catechins and *cis*-epicatechins (Fig. 1). Each of geometric isomer, in turn, exists as two optical isomers: (+)-catechin and (−)-catechin (C) and (+)-epicatechin and (−)-epicatechin (EC), respectively. (−)-Catechin can be modified by esterification with gallic acid to form (−)-catechin-3-gallate (CG), epicatechin-3-gallate (ECG), (–)-epigallocatechin-3-gallate (EGCG), and (−)-gallocatechin-3-gallate (GCG), respectively. Four theaflavins (theaflavin (TF), theaflavin-3-gallate (TF3G), theaflavin-3′-gallate (TF3′G), and theaflavin-3,3′-digallate (TF33′G)] (Fig. 1)

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are formed postharvest by enzyme-catalyzed oxidative dimerization of catechins.

The main objective of this review is to unify and interpret widely scattered information of reported studies on inhibitory activities of tea flavonoids and teas against pathogenic bacteria, bacterial toxins produced by some of these bacteria, and pathogenic and phytopathogenic viruses and fungi. It should be noted that polyphenolic "flavonoids" from plant sources other than tea also possess antimicrobial properties [13]. The information and suggested research outlined below may facilitate and guide further needed studies to optimize the use of tea compounds and teas in order to improve microbial food safety as well as to prevent or treat infectious diseases of animals and humans.

General comments in the text concerning pathogenicity are based on information listed in the Merck Manual of Diagnosis and Therapy [14] and in the PDR Medical Dictionary [15].

2 Factors that may influence the flavonoid content of tea leaves

Commercial tea leaves provide a rich source of dietary flavonoids [16]. The literature suggests that geographical origin, soil composition, differences in the composition of different leaves, time of harvesting, postharvest treatments, and physical structure of the different leaves probably influence the composition of tea leaves. Also contributing to this variability is the susceptibility of tea compounds to extraction by different solvents [17–19] (Fig. 2). For example, we recently reported that significantly greater quantities of individual and total flavonoids (catechins and theaflavins) were extracted with 80% ethanol/water at 60°C for 15 min than with boiled water for 5 min from 77 commercial teas sold in the United States [20, 21]. The latter conditions are widely used at home to prepare tea infusions. The distribution of the individual catechins and theaflavins in individual teas extracted by the two solvents also varied. The following ranges of concentrations of flavonoids (catechins plus theaflavins) in the tea leaves extracted with 80% ethanol were observed (in mg/g): in 32 black teas, 19.8–115.1; in 24 green teas, 12.3–136.3; and in 14 specialty teas, 4.9–118.5. These results make it possible to maximize the extraction of tea compounds for antimicrobial studies and to better relate the content of flavonoids and alkaloids of teas and dietary supplements to their health-promoting effects.

A striking example of the influence of environmental factors on tea composition and antimicrobial activities is the observation by Chou *et al.* [22] that the antibacterial activities of teas against *Ba. cereus*, *E. coli*, *Proteus vulgaris*, *Pseudomonas fluorescence*, *Salmonella* spp. and *St. aureus* are influenced both by harvesting (manufacturing) season and the extent of postharvest fermentation in the following order: green tea > oolong tea > black tea. Extracts of oolong tea leaves prepared in the summer exhibited the strongest activity followed by extracts prepared in the spring, winter, or fall (Fig. 3A). The data suggest that antibacterial activity of teas is related to the flavonoid levels, which in turn are influenced by the degree of fermentation and harvesting season.

The cited studies may offer a possible explanation for the variable antimicrobial results obtained by different investigators with teas from different sources, discussed below.

3 Antibacterial activities of tea flavonoids and teas

This section presents brief overviews of reported studies on tea catechins' and teas' inhibition of growth and/or destruction of a variety of pathogenic bacteria that can cause human illness. These pathogens are listed in alphabetical order.

3.1 *Ba. cereus*

Ba. cereus is a widely distributed foodborne pathogen that causes vomiting and diarrhea in mammals including humans. Previously, we evaluated the antimicrobial activities of seven green tea catechins and four black tea theaflavins as well as aqueous extracts (infusions) of 36 commercial black, green, oolong, white, and herbal teas against one *Ba. cereus* strain [3]. The results showed that (i) GCG, EGCG, CG, ECG, TF33′G, TF3′G, and TF3G exhibited antimicrobial activities at nanomolar levels; (ii) some flavonoids were more active than medicinal antibiotics such as tetracycline or vancomycin at comparable concentrations; (iii) bactericidal activities of the teas could be roughly accounted for by the levels of catechins and theaflavins determined by HPLC (Fig. 2); (iv) freshly prepared tea infusions were more active than day-old teas; and (v) tea catechins without gallate side chains, gallic acid and the alkaloids caffeine and theobromine also present in teas, and herbal (chamomile, peppermint) teas which contain no flavonoids were all inactive. Although *Ba. cereus* is a spore-forming bacterium, sporulation was not apparent in the course of these studies.

Other investigators [23] reported inactivation of *Ba. cereus* by micromolar concentrations of tea compounds compared to nanomolar levels observed in our study. These observations suggest that different strains of the same organism can exhibit different susceptibilities to inactivation by tea flavonoids.

3.2 *Ca. jejuni*

Ca. jejuni is a widely distributed foodborne pathogen that causes gastroenteritis and diarrhea in humans and abortions in sheep. There is also an apparent association between outbreaks of *Campylobacter*-induced diarrhea and the subse-

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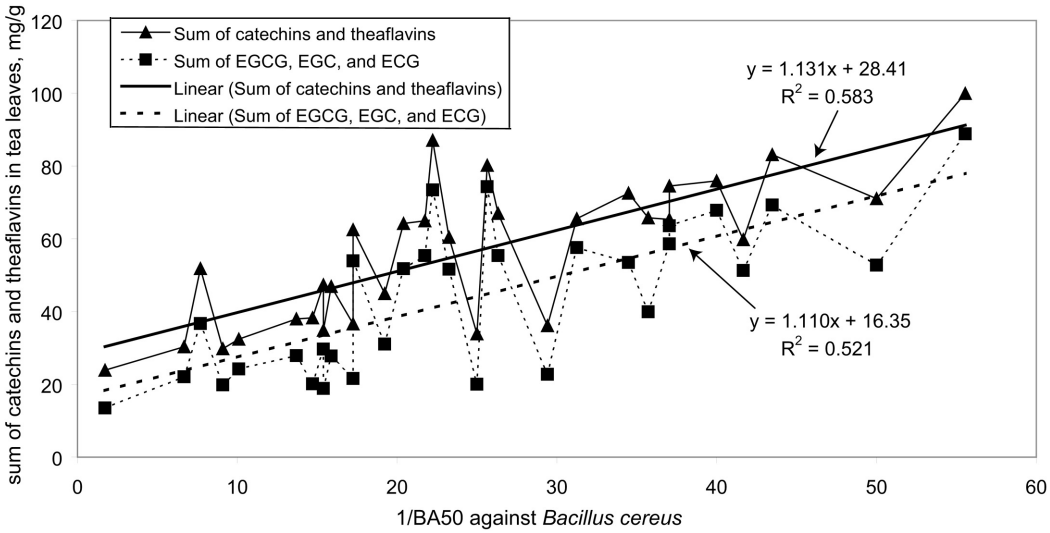

Figure 2. Relationship between the flavonoid content of 36 teas and their bactericidal activities against *Ba. cereus* (adapted from Friedman *et al.* [3]).

quent development of Guillain-Barré syndrome in humans [14]. Extracts of black and green tea inhibited the growth of clinical isolates of *Ca. jejuni* and *Ca. coli* within 4 h [24].

3.3 *Cl. perfringens* and other spore-forming bacteria

The toxin-producing, spore-forming foodborne pathogen *Cl. perfringens* causes gastroenteritis in humans following ingestion of contaminated meat. Studies by Hara *et al.* [25, 26] and by Ahn *et al.* [27] showed that tea catechins strongly inhibited the organism *in vitro*. Green tea catechins also reduced the heat-resistance of the spore-forming thermophilic spoilage bacteria *Ba. stearothermophilus* and *Cl. hermoaceticum*, which proliferate in vending machines, causing sour spoilage in milk and other drinks (Fig. 3B) [28]. Juneja *et al.* (submitted) found that a concentrated green tea extract inhibited sporulation and growth of *Cl. perfringens* in ground meat and turkey products during chilling. These observations suggest that the use of polyphenols or other natural antimicrobials may result in reduction of temperatures used in thermal processing of foods. Because widely consumed well-done meat products exposed to high cooking temperatures employed to kill pathogens also induce the formation of potentially carcinogenic heterocyclic amines [29, 30], reduction in cooking temperatures is a highly desirable objective. Spore-forming toxin-producing *Ba. anthracis* and *Ba. botulinum* are discussed below in Section 8.

3.4 *E. coli* O157:H7 and related pathogens

E. coli O157:H7 is a foodborne, toxin-producing enteropathogen responsible for a hemorrhagic form of colitis, bloody diarrhea, and hemolytic uremic syndrome. The toxins are related to those formed by *Shigella*, *Cholera*, and other enteropathogens. Here we briefly review reported activities of tea compounds and teas against this organism *in vivo* and *in vitro*. The following observations are relevant to the theme of this paper:

(i) A green tea extract protected mice against neurologic and systemic symptoms caused by infection with *E. coli* O157:H7 [31]. The level of Shiga-like toxins (STN) in the feces of mice on the tea diet was lower than on the control diet. A subsequent study showed that a green tea extract exhibited *in vivo* synergy with the antibiotic levofloxacin against infection of mice by *E. coli* O157:H7 [32].

(ii) A green tea extract had a wide spectrum of activity against 30 different pathogenic bacteria including strains of *E. coli* [33, 34].

(iii) A methanol extract of tea leaves protected Swiss white mice against mortality caused by *Sa. typhimurium* [35]. The protective effect was accompanied by significant reduction in the blood levels of the bacteria. The extract was also active *in vitro* against many Gram-positive and Gram-negative bacteria.

(iv) The average minimum inhibitory concentration (MIC) values (in μg/mL; the lower the value the greater the activity) of a series of plant polyphenols including catechins and theaflavins against *St. aureus* and the genus *Vibrio* (192 ± 91 and 162 ± 165, respectively) were much lower than the corresponding values against the genus *Salmonella* (795 ± 590) and *E. coli* (1519 ± 949) [36].

(v) Si *et al.* [37] describe a bioassay-guided antimicrobial assay of tea components against *E. coli* O157:H7 and other bacteria.

(vi) Although tea extracts were active against *St. aureus* and *Li. monocytogenes in vitro*, they were inactive against these organisms in beef as well as against *E. coli* O157:H7 *in vitro* [38]. These results suggest that *E. coli* O157:H7 may be less susceptible to inactivation by flavonoids than

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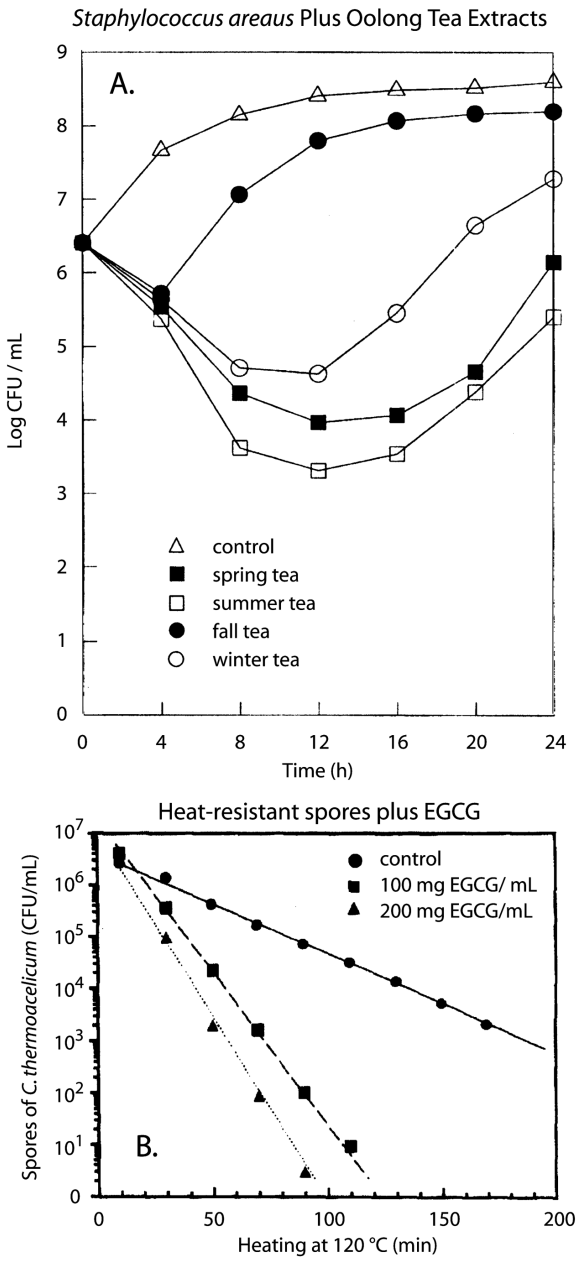

Figure 3. (A) Antimicrobial activities of oolong teas vary with harvest season (adapted from Chou *et al.* [22]). (B) Epigallocatechin-3-gallage facilitates heat inactivation of bacterial spores (adapted from Sakanaka *et al.* [28]).

are other pathogenic bacteria. It also implies that binding of flavonoids to meat proteins may prevent them from interacting with the bacteria. This aspect merits further study.

3.5 *Helicobacter pylori*

H. pylori is a urease producing gastric pathogens that may contribute to the formation of ulcers and to low-grade gastric lymphoma in humans. Tea compounds have been extensively evaluated for their ability to inhibit this organism [39]. These include the following observations:

(i) Studies by Yee *et al.* [40] and Yee and Koo [41] revealed an inverse relationship between Chinese tea consumption and *Helicobacter* infection. The authors note, however, that because Chinese and Japanese people have high rates of gastric cancer and ulcers, other factors including hygiene and nutrition may impact *Helicobacter* infection.

(ii) Yanagawa *et al.* [42] noted additive effects of the antibiotic amoxicillin and EGCG against nonresistant and antibiotic-resistant clinical isolates of *H. pylori*. The authors suggest that ECGC merits further evaluation in the therapy of both nonresistant and antibiotic-resistant *H. pylori* infections and associated symptoms.

(iii) Pretreatment with ECGC protected gastric mucosa epithelial cells against *H. pylori*-induced apoptotic cell death and DNA damage [43]. The mechanism of this beneficial effect appears to involve blockage of activation of cellular signaling pathways. This would result in reduced synthesis of the proinflammatory mediator, hydroxyeicosatetraenoic acid. Inactivation of the VacA toxin produced by *H. pylori* is mentioned in Section 8.

3.6 *Legionella pneumophila*

Le. pneumophila, a bacterium found in soil and in stagnant water-storage containers, causes Legionnaires' disease, an infection of the lungs and other organs. ECGC enhanced the *in vitro* resistance of alveolar macrophages to infection by *Le. pneumophila* [44, 45]. The mechanism of the protective effect appears to involve selective immunomodulatory action of catechin on cytokine formation. The protective effect was also apparent against tobacco smoke-induced impairment of alveolar macrophages. The authors suggest that EGCG may benefit heavy smokers.

3.7 *Mycobacterium tuberculosis*

Mycob. tuberculosis is a pathogenic bacterium that causes tuberculosis in humans. Anand *et al.* [46] found that dose-dependent down-regulation (inhibition) of TACO gene expression by EGCG was accompanied by inhibition of the survival of *Mycob. tuberculosis* within host macrophages. The authors suggest that the tea catechin could contribute to the prevention of tuberculosis infection.

3.8 *Mycoplasma pneumoniae*

Mycop. pneumoniae is a group of prokaryotic microorganisms lacking a cell wall, considered to be different from bacteria or viruses. They cause primary atypical pneumonia in humans. Chosa *et al.* [47] found that at concentrations of 0.2%, both green and black tea exhibited bactericidal activities against *Mycop. pneumoniae* organisms, and that ECGG isolated from green tea and theaflavin from black tea

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showed marked activity against *Mycop. pneumoniae* as well as against *Mycop. orale* and *Mycop. salivarum* found in saliva, associated with buccal cavities. These observations suggest that tea flavonoids may be useful in the prophylaxis and treatment of pneumonia and infections of the oral cavity further described below under oral pathogenic bacteria.

3.9 Ocular pathogenic bacteria

Pathogenic bacteria that infect the eyes produce high amounts of gelatinases. ECGC inhibited gelatinase activity produced by several strains of ocular pathogens with an IC_{50} value of ~200 μM [48]. The inhibition can delay the invasive spread of the bacteria in the eyes that thrive on a gelatin substrate.

3.10 Oral pathogenic bacteria

Several studies describe anticariogenic/cariostatic effects of tea compounds. These include the following observations:

(i) Dental caries is the most common infectious disease affecting humans [49]. The main causes are a group of acid-producing *Streptococci* referred to as mutans streptococci. *Str. mutans* and *Str. sobrinus* are reported to be the major infective agents of human dental plaque.

(ii) Salivary amylase hydrolyzes food starch to sugars (maltose, glucose) that are then fermented by bacterial enzymes in oral cavities to caries-inducing organic acids. Both black and green tea infusions inhibited salivary amylase and the consequent intraoral hydrolysis of starch in human volunteers [50]. Another study with human volunteers [51] showed that rinsing of the mouth with 2 mg/mL EGCG solution followed by a 10% sucrose solution 30 min later prevented lowering of pH induced by cariogenic bacteria.

(iii) *In vivo* and *in vitro* experiments showed that a green tea extract inhibited caries formation in hamsters and increased the resistance of human enamel to acid [52]. A high molecular weight, nondialyzable component of the green tea appears to be responsible for the observed beneficial effects. A related study showed that a black tea extract fed to inbred hamsters decreased caries formation by 56.6% on a regular diet and by 63.7% on a cariogenic diet [53]. The authors suggest that frequent consumption of black tea may significantly decrease caries formation, even in the presence of sugars in the diet.

(iv) Tea polyphenols (1–4 mg/mL) strongly inhibited attachment of *Str. mutans* and other bacteria to collagen *in vitro* [54]. Related studies indicated that oolong tea extracts and tea polyphenols also inhibited acid production by mutans streptococci as well as by *Actinomyces viscosus* that are part of the oral microflora [55, 56]. These observations suggest that tea polyphenols may prevent dental caries by inhibiting bacterial adherence to tooth surfaces and by reducing levels of food-derived acids that can damage the tooth enamel.

(v) Consumption of black tea by caries-prone young rats fed a cariogenic-diet for 2 wk reduced the development and progression of caries [57].

(vi) Yun *et al.* [58] describe inhibitory effects of EGCG at 20 μM levels on expression of matrix metalloproteinase-9 and on the formation of osteoclasts associated with periodontal diseases. This finding suggests that ECGC may prevent alveolar bone resorption that occurs in periodontal gum diseases.

The above-cited observations suggest that both green and black tea flavonoids inhibit dental caries in animals and humans. Specifically, the published data show that tea can indeed reduce the cariogenic potential of starch-containing foods by suppressing the release of fermentable carbohydrates in the oral cavities of animals and humans and also by reducing the levels of organic acids in oral cavities, thus enhancing dental health. Polymeric black tea flavonoids (thearubigins?), appear to exhibit the highest anticariogenic effects by inhibiting attachment of cariogenic bacteria to tooth surfaces and gums and by inhibiting enzymes (salivary amylase, lactate dehydrogenase) that catalyze the eventual formation of compounds that lower the pH in oral cavities.

3.11 *Salmonella* spp.

Sa. typhi species cause typhoid fever, *Sa. paratyphi* cause enteric fever, and *Sa. typhimurium* causes food poisoning in humans. *Sa. typhi* bacteria showed greater susceptibility to inhibition by a alcoholic extracts of black tea than did *Sa. paratyphi* bacteria [59]. These bacteria are serotypes of *Sa. enterica* subsp. *enterica*.

3.12 Spoilage bacteria

A green extract inhibited the growth of several species of bacteria known to adversely affect the quality of some foods, the so-called spoilage organisms [33]. These include *Pr. vulgaris*, *Ps. aeruginosa*, and *Serratia marcescens*. These results suggest that tea compounds possess the potential of enhancing food quality as well as safety.

3.13 *St. aureus*

St. aureus is a highly pathogenic, Gram-positive, aerobic, toxin-producing, foodborne organism that can contaminate food and infect the skin, lung, heart, and other organs. The bacterium causes foodborne diseases worldwide [60]. A tea extract, ECGC, or theaflavin digallate inhibited the growth of methicillin-resistant *St. aureus* strains in culture [61]. Higher levels (MIC = 800 μg/mL) were needed to inhibit Gram-negative rods (*E. coli*, *Klebsiella pneumoniae*, *Sa. typhi*, *Pr. mirabilis*, *Ps. aeruginosa*, and *Se. marcescens*) compared to MIC concentrations of 50–100 μg/mL of EGCG against several strains of *Staphylococci* (*St. aureus*,

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St. epidermis, *St. hominis*, and *St. haemolyticus*) [62]. The authors suggest that the structure of the cell wall as well as the variable affinities of ECGC to cell wall components (peptidoglycans) may govern the various susceptibilities of Gram-positive and Gram-negative bacteria to EGCG. The enterotoxin produced by *Staphylococci* is mentioned below under bacterial toxins.

These cited observations show that tea catechins, theaflavins, and tea extracts containing both classes of polyphenolic compounds exhibited strong antibacterial activities against foodborne pathogenic bacteria, food spoilage bacteria, and pathogenic bacteria that cause infectious illnesses in humans. The available information provides a basis for needed studies on the potential of the tea compounds to inactivate bacteria in liquid and solid foods and to protect humans against infectious diseases. The latter aspect is examined in more detail below.

4 Synergy of combinations of catechins and medical antibiotics

The following observations show that some combinations of catechins with medical antibiotics exhibit synergistic activities and/or were effective against antibiotic-resistant bacteria.

(i) Aqueous tea extracts inhibited methicillin-resistant *St. aureus* as well as a wide range of nonresistant pathogenic organisms [34]. ECG converted a methicillin-resistant phenotype to a methicillin-sensitive one.

(ii) Combinations of EGCG and carbapenem antibiotics exhibited synergistic activities against clinical isolates of methicillin-resistant *St. aureus* [63].

(iii) EGCG synergizes the activity of β-lactam antibiotics against *St. aureus* by binding to the peptidoglycan component of the bacterial cell wall [64].

(iv) Theasinensin A, a decomposition product of EGCG, prevented antibiotic resistance of methicillin-resistant *St. aureus* [65].

(v) ECG was more effective in modulating β-lactam antibiotic resistance in *St. aureus* than ECGC [66, 67]. Nongalloylated catechins also potentiated the activity of oxacillin against *St. aureus*.

(vi) EGCG at doses MIC values of <100 μg/mL reversed resistance of *Staphylococci* to tetracycline [68]. The beneficial effect of EGCG at the cellular-molecular level appears to be due to increased intracellular retention of tetracycline and may be associated with the inhibition of the expression of efflux pump proteins.

(vii) Combinations of green tea with butylated hydroxyanisole were more effective against bacteria and fungi than green tea alone [69].

(viii) A combination of catechins and the antibiotic ciprofloxacin acted synergistically to alleviate chronic bacterial prostatitis in rats [70].

(ix) Combinations of various teas with gentamicin, methicillin, and nalidixic acid acted synergistically against *Shigella dysenteriae* [71].

These cited beneficial effects of combinations of flavonoids and medicinal antibiotics suggest the need to ascertain if such combinations are also effective in the prevention and therapy of human infections. If they are indeed effective, patients would be exposed to lower levels of antibiotics, thus minimizing any side effects and arresting or delaying development of antibiotic-resistance.

5 Mechanistic aspects

Catechins regulate expression of the gene(s) coding for cytochrome P450. They also regulate the expression of the gene(s) for inflammatory cytokine TNF-α. This results in amelioration of clinical symptoms of *E. coli* infection [72, 73]. Detailed physicochemical studies suggest that the bactericidal activities of galloylated tea catechins at the cell membrane level may be due to their specific perturbations of the ordered structure of phosphatidylcholine and phosphatidylethanolamine bilayers constituting bacterial cell wall membranes [74, 75]. ECGC was found to be the most effective catechin in perturbing the membrane structure of bacteria-like model membranes, causing leakage from *E. coli*-isolated membranes. Differential effects of catechins on bacterial cell walls compared to membranes of human cells may be due to differences in structures of the respective walls (membranes).

EGCG can inhibit penicillinase, an enzyme that degrades penicillin [76]. The bactericidal action of EGCG may depend on hydrogen peroxide derived from the reaction EGCG with oxygen (prooxidative activity) [77, 78]. These observations suggest that antimicrobial effects arise from the interactions of catechins with oxygen, genes, cell membranes, and enzymes.

6 Phytopathogens

Phytopathogenic bacteria such as strains of *Agrobacterium*, *Clavibacter*, *Pseudomonas*, *Erwinia*, and *Xanthomonas* contaminate produce (eggplants, grapes, cabbage, lettuce, onions, potatoes, tomatoes). ECG and ECGC as well as theaflavins inhibited the growth of these bacteria in culture at MIC values of ~100 ppm [79]. Related studies showed that EGCG, ECG, and theaflavins bound to and inactivated tobacco and cucumber mosaic viruses that cause lesions in plant leaves [80, 81]. The authors suggest that further studies are needed to determine the effectiveness of the tea compounds against canker (soft rot), wilt, and other necrotic plant diseases caused by the phytopathogens on fruits and vegetables.

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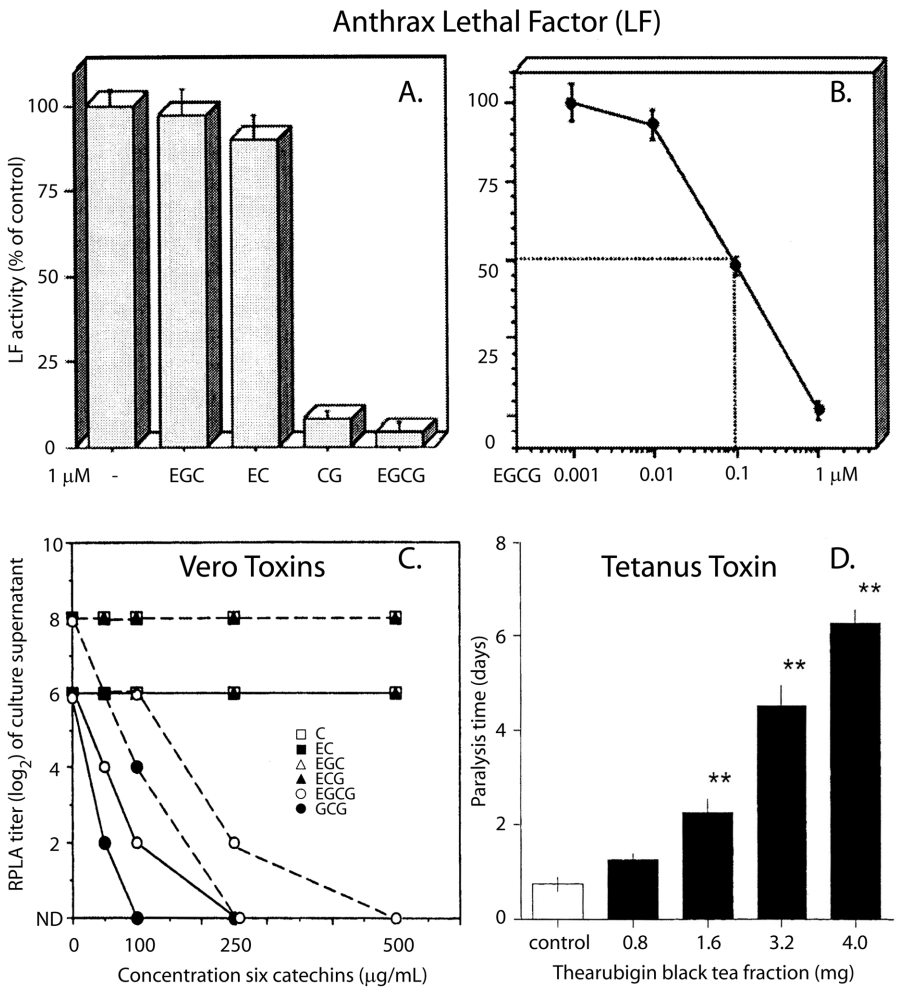

Figure 4. (A) Inhibition of the anthrax LF by four catechins (adapted from Dell'Aica *et al.* [84]). (B) Concentration dependence of the inhibition of the anthrax LF by epigallocatechin-3-gallate (adapted from Dell'Aica *et al.* [84]). (C). Structure–activity relationships of catechin-induced inhibition of release of the Vero toxin from *E. coli* O157:H7 (adapted from Sugita-Konishi *et al.* [108]). (D) Inactivation of the tetanus toxin by polymeric black tea thearubigins (adapted from Satoh *et al.* [88]).

Because tea flavonoids may protect plants against phytopathogens as well as against human pathogens, similar mechanisms may govern the inhibition of both pathogen types. This aspect merits further study.

7 Miscellaneous aspects

Not only flavonoids but also volatile components of green-tea flavor inhibited both pathogenic as well as spoilage bacteria [82]. Yao *et al.* [83] developed an analytical sensor instrument that may be suitable to determine antimicrobial properties of teas and Si *et al.* [37] describe a bioassay-guided procedure for the identification of antimicrobial tea components in tea extracts. The method was used to demonstrate a direct influence of tea composition on antimicrobial activities of teas.

8 Inactivation of protein toxins by tea compounds and teas

Most of the known bacterial and snake venom toxins are proteins whose virulence is determined by their 3-D conformations. Thus, alteration of the native structural integrity of these proteins should inactivate them by preventing the *in vivo* molecular interactions with cell membrane receptor sites of their hosts and hosts of other biomolecules that the bacteria need to survive. There is a need to develop food-compatible conditions to alter the structures of bacterial

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and plant protein toxins, thus transforming toxic proteins to nontoxic ones. Below are outlined some reported efforts to inactivate protein toxins with the aid of tea catechins and theaflavins.

8.1 Anthrax toxin

Infection of human skin by spore-forming, toxin-producing *Ba. anthracis* bacteria causes a severe disease characterized by septicemia and hemorrhage. ECGC has been reported to strongly inhibit the anthrax lethal factor (LF) produced by *Ba. anthracis*, with an IC_{50} (concentration of the catechin that inhibited 50% of the toxin) of 97 nM (Figs. 4A, B) [84]. The catechin prevented not only toxin-induced death of macrophages but also resulted in the survival of the Fischer 344 rats. Inactivation of the anthrax toxin may result from binding of phenolic OH groups of the (–)-epicatechin-3-gallate to the zinc atom associated with the metalloproteinase of the toxin [85, 86] and/or to antioxidative effects of the catechin [87]. It is not known whether theaflavins will also inhibit the anthrax toxin.

8.2 Botulinum neurotoxins

Ingestion of food contaminated by neurotoxin-producing *Cl. botulinum* spore-forming bacteria causes the disease botulism, characterized by paralysis due to blocking of motor nerve terminals at the myoneural junction. The thearubigin polymeric fraction of black tea blocked the neuromuscular action of botulinum neurotoxins A, B, and E produced by *Cl. botulinum* in mouse phrenic nerve-diaphragm preparations [88, 89]. The antitoxin effect appears to result from covalent binding (chelation) of the catechin to the metalloproteinase part of the toxin. Kaempfenol, kaempferol, and quercetin glycosides isolated from black tea also inhibited toxic manifestations of the botulinum neurotoxin [90, 91]. Figure 5 depicts sites on the toxin molecule susceptible to inactivation.

8.3 Cholera toxin

Ingestion of drinking water or cooked shellfish contaminated by the bacterium *Vibrio cholerae* causes the potentially fatal disease cholera, characterized by profuse diarrhea. Diarrhea results from the interaction of the cholera enterotoxin with adenylate cyclase of the mucosa of the digestive tract, causing water flow from the open ion channels through osmosis. Toda *et al.* [92, 93] found that tea catechins protected against experimental infection by *V. cholerae* O1 bacteria.

Other polyphenolic compounds also inhibited the virulence of the cholera toxin [94]. Using a different approach, Shimamura *et al.* [95] found that SH-containing compounds such as cysteine and reduced glutathione inhibited the production of cholera toxin by *V. cholerae* and that added vitamin B_{12} reversed the inhibition. These observations suggest that inhibition results from the formation of –S–S– bond between added and toxin SH groups *via* sulfhydryl-disulfide interchange by mechanisms described in detail elsewhere for the inactivation of soybean inhibitors of digestive enzyme [96–98] (see also Fig. 5).

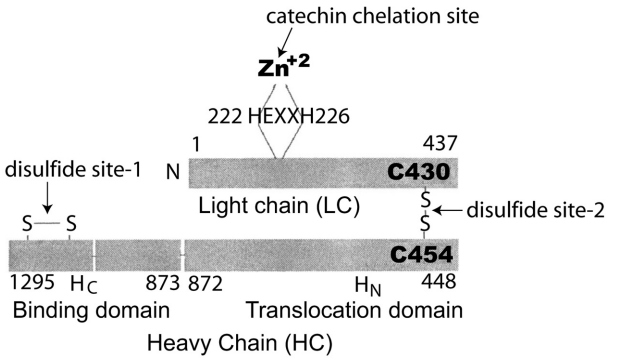

Figure 5. Structure of botulinum neurotoxin [152] showing three potential sites for inactivation: 1, zinc-containing metalloproteinase susceptible to chelation by catechin phenolic OH groups; 2, intramolecular disulfide bond of the heavy chain; 3, intermolecular disulfide bond linking the light and heavy chains. The disulfide bonds are susceptible to reduction and/or sulfhydryl-disulfide interchange initiated by sulfhydryl compounds such as *N*-acetyl-L-cysteine.

8.4 *Helicobacter* VacA toxin

The *H. pylori*-vacuolating cytotoxin, VacA, may be largely responsible for the gastritis and ulceration mentioned above. Yahiro *et al.* [99] found that oral administration of a high-molecular weight polyphenolic compound extracted from hop bracts was more effective than other phenolic compounds in inhibiting VacA-induced gastritis in mice. The antitoxin effect may result from the formation of a complex between the toxin and the polyphenol and/or to inhibition of binding of the toxin to cell receptors in the digestive tract.

8.5 Pertussis toxin

Virulent strains of *Bordella pertussis* produce a protein exotoxin that is activated after binding to membrane receptors of the larynx, trachea, and bronchi. The organism causes the disease pertussis (whooping cough), characterized by spasmodic coughing. Low concentrations of tea leaf catechins were more effective than formalin in detoxifying pertussis toxin produced by *Bo. pertussis* [100, 101]. Mice immunized with a vaccine prepared with catechins were protected against infection by the pathogenic bacteria. EGCG and TF3 inactivated leucolymphcytosis promoting activity of the pertussin toxin [102].

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8.6 Snake venom toxins

Intraperitoneal injection of 3 mg of a "melanin" extracted from black tea substantially reduced the toxic effects of several snake venom toxins [103]. The antivenin activity appears to be due to the chelation of the tea melanin to calcium ions and to nonspecific binding to phospholipase A2 present in the snake venom. The structure of the melanin, possibly a polymeric thearubigin, has not yet been defined.

8.7 Staphylococcal enterotoxin B (SEB)

Intraperitoneal administration of a green tea extract and of ECGC to BALB/c mice bound to and inhibited the SEB [104]. Inhibition of the heat-resistant enterotoxin was both dose- and time-dependent. ECGC also inhibited *Staphylococcal* superantigens (SsAgs)-induced activation of T-cells both *in vitro* and *in vivo*. Since these antigens aggravate atopic dermatitis, the authors suggest that catechins may be useful in the treatment of this human disease.

8.8 Tetanus neurotoxin

Spores of *Cl. tetani* infesting a wound release the virulent neurotoxin tetanospasmin after germination. Tetanospasmin acts on the central nervous system, resulting in muscular contraction that may result in death unless the subject has been previously vaccinated. The polymeric thearubigin fraction of black teas protected against the toxin-induced onset of paralysis in mouse phrenic nerve-diaphragm preparation (Fig. 4D) [88]. The antitoxin effect appears to result from covalent binding of thearubigin to the toxin, analogous to the protective effect of thearubigin against the botulinum toxin mentioned above. Black, oolong, and roasted tea infusions, but not green tea, also protected against tetanus toxin toxicity [105, 106].

8.9 Vero (Shiga) toxins

Enteric disease-causing pathogenic *E. coli* O157:H7 (and other *E. coli* and *Shigella* strains), transmitted largely *via* the food chain, produce so-called Shiga (Vero) toxins. Intraperitoneal administration of 1 mg of EGCG to BALB/c mice completely inhibited the lethal effect of 2 ng of the Vero toxin (VT2) produced by *E. coli* O157:H7 [107]. Catechin (50 μg/mL) was also bactericidal after a 24 h exposure. EGCG and GCG also markedly inhibited the extracellular release of a Vero toxin from *E. coli* O157:H7 (Fig. 4C) [108]. The mechanism of inhibition appears to involve interference by the catechins of the transfer of periplasmic proteins through the outer membrane of the bacterial cell. Inhibition of leakage of toxin proteins from the periplasm contrasts with mechanisms of the bactericidal effect proposed by Ikigai *et al.* [109] which involves disruption of the cell membrane, resulting in leakage of molecules essential for the viability of the bacteria. Thiols and disulfide compounds reduced the secretion in suckling mice of an enterotoxin produced by *E. coli* [110].

The cited findings indicate that green tea catechins and their polymeric oxidation products, known as thearubigins present in black teas, are potent inhibitors of several virulent bacterial toxins. A largely unanswered question is whether tea compounds and teas can inactivate bacterial toxins present in drinking water and in liquid and solid foods.

9 Antiviral activities of tea flavonoids and teas

Viral contamination of food and infection of animals and humans is a major cause of numerous pandemic diseases. The mechanism of antiviral action of polyphenolic compounds is based on their abilities to act as antioxidants, to inhibit enzymes, to disrupt cell membranes, to prevent viral binding and penetration into cells, and to trigger the host cell self-defense mechanisms. Below we present a brief overview of reported studies on the antiviral activities of green tea catechins and black tea theaflavins against several pathogenic viruses.

9.1 Adenovirus

Adenovirus, also known as adenoidal-pharyngeal-conjuctival (APC) virus, infects the mucous membranes of the respiratory and urinary tracts as well as the linings of the eyes and intestine causing conjunctivitis, gastroenteritis, and other symptoms. Tea catechins and tea infusions inhibited both adenovirus infection and the viral protein adenain in HepG2 cells (Fig. 6A) [111]. The determined high therapeutic index (ratio of antiviral to cytotoxic dose) of 22 for ECGC suggests that this catechin may be safe to treat adenovirus infections in humans.

Because the virulent adenain protein appears to be the main target of the catechins in preventing infection, expectations are that catechins will also inactivate other adenoviral strains containing this protein.

9.2 Bacteriophages

Virulent bacteriophages are viruses that have specific affinities for bacteria. They induce lysis of the bacteria they infect. Infusions from nine types of teas inactivated the virulent bacteriophages Felix 01 and P22, without affecting the infected *Salmonella* [112].

9.3 Bovine coronavirus and bovine rotavirus

These viruses cause diarrhea and gastroenteritis in calves and cattle, resulting in significant losses to agriculture. A mixture of four theaflavins isolated from black tea had a significantly higher (synergistic) antirotaviral activity than did the sum of activities of the four individual compounds [113]. The crude tea extract was also active against the cor-

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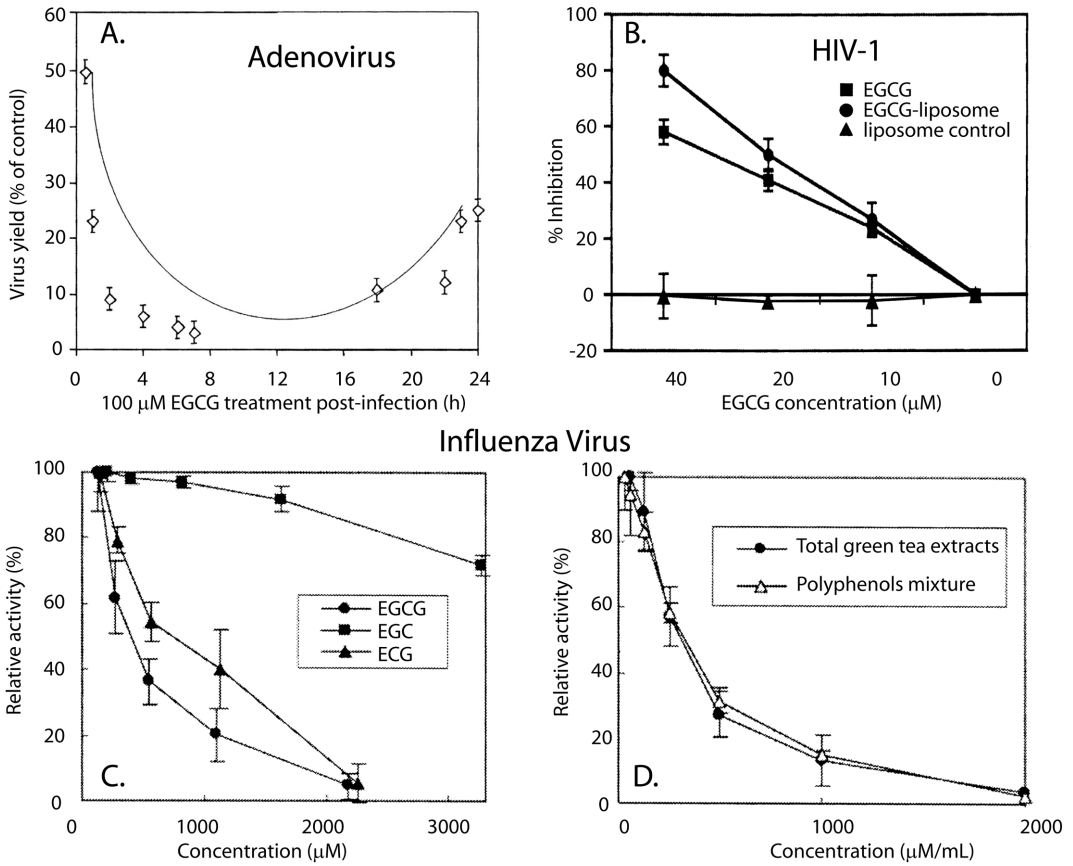

Figure 6. (A) Time-dependence of the inhibition of the adenovirus by epigallocatechin-3-gallate (adapted from Weber *et al.* [111]). (B) Concentration dependence of the inhibition of HIV-1 by epigallocatechin-3-gallate (adapted from Liu *et al.* [118]). (C) Concentration dependence of the inhibition of the influenza virus by three catechins (adapted from Song *et al.* [122]). D. Concentration dependence of the inhibition of the influenza virus by green extracts (adapted from Song *et al.* [122]).

onavirus. Molecular modeling of the structures of the four theaflavins indicates that steric and conformational effects appear to govern viral infectivity.

9.4 Epstein-Barr virus (EBV)

Infections by EBV, also known as human herpesvirus 4, may cause infectious mononucleosis and may be linked to the causes of Burkitt's lymphoma and nasopharyngeal carcinoma [14]. EGCG (50 μM) inhibited the expression of EBV lytic proteins [114]. The proposed mechanism of the inhibition appears to involve inhibiting transcription of early genes that govern the initiation of the EBV lytic cascade. The authors suggest that EGCG has the potential to treat viral infections.

9.5 Herpes simplex virus (HSV-1)

The HSV, also known as cold sore, causes blisters on the mouth and lips (orofacial infections) and on genitals. A study by Savi *et al.* [115] of structure–antiherpetic/genotoxic activities of six tea catechins showed that (i) all compounds exhibited antiviral activities with selective indices ranging from 1.3 to 13; (ii) concentrations of catechins that protected Vero cells against viral infection also induced genotoxicity determined by the Comet assay; and (iii) the number of OH groups on the B ring of the catechin molecule as well as the absence or presence of galloyl side chains influenced the ratio of the antiviral to DNA-damaging effects.

Prodelphinidin B-2 3′-gallate isolated from green tea leaves also exhibited anti-HSV type 2 activities *in vitro*, with an IC_{50} value of 5.0 μM [116]. The proposed mechanism governing the antiviral effect of the proanthocyanidin involves inhibition of viral attachment to Vero cells and penetration and disturbance of the late stage of viral infection.

Because potato glycoalkaloids are also reported to exhibit antibiotic, including antiviral effects against the herpes virus (reviewed in [117]), the antiherpes virus effects of concurrent consumption of both potatoes and teas in humans merits further study. The cited results can guide selecting food ingredients with high antiviral activities.

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9.6 Human immunodeficiency virus type 1 (HIV-1)

HIV is a retrovirus that causes the widespread human acquired immunodeficiency syndrome (AIDS). Black tea theaflavins were more effective in inhibiting HIV-replication than green tea catechins [118]. The polyphenolic compounds inhibited HIV-1 entry into target cells by blocking HIV-1 envelope glycoprotein-mediated membrane fusion. These and additional observations on the anti-HIV activities of tea compounds and their complex mechanisms of action at the molecular and cellular levels (Fig. 6B), including those by Yamaguchi *et al.* [119] and Hamza and Zhan [120], suggest that both black and green teas and their isolated constituents may contribute to the preventive and therapeutic armamentarium against HIV infection in humans.

9.7 Influenza virus

Influenza is an infectious human disease caused by a multiplicity of influenza viruses. ECGC prevented infection by the influenza virus by binding to the viral hemagglutinin [121]. The bound viral particles cannot attach to the target receptor cells. Related cell culture studies showed that changes of viral membrane properties contributed to the antiviral effect of tea catechins against the influenza virus [122]. EGCG and ECG were found to be 10–15 times more active against the influenza virus than EGC (Figs. 6C and D). These results show that the 3-galloyl side chain of the catechins potentiates antiviral activity of the parent catechin molecule.

The cited observations suggest that knowledge of structural requirements for antiviral activities of tea and related plant compounds may enable applications of the most active compounds to help protect plants, food, animals, and humans against adverse effects of pathogenic viruses.

10 Antifungal activities

EGCG, theaflavin digallate, and tea extracts exhibited variable time- and concentration-dependent fungicidal activities against several fungi [123]. More detailed studies by Hirasawa and Takada [124] revealed that (i) the antifungal activities of tea catechins against the opportunistic fungus *Candida albicans* was greater at pH 7 than at pH 6 or 6.5; (iii) ECGC enhanced the antifungal activity of the drug amphoterecin B; and (c) the combined use of ECCG and the antifungal drug fluconazole inhibited fluconazole-resistant strains of this fungus. These results suggest that the combined use of catechins and antifungal drugs may be useful in the treatment of *Can. albicans* superinfections of the oral cavities, intestine, and vagina resulting from over consumption of antibiotics. Table 1 lists the pathogenic bacteria, toxins, viruses, and fungi discussed in the text or mentioned in the cited references.

11 Bioavailability of flavonoids – human studies

A journal reviewer raised the following questions: "Have studies been carried out in humans that demonstrate inhibition of bacteria by tea"? Are inhibitory effects demonstrated by tea extracts clinically significant, *i.e.*, would they be expected to impact on symptoms"? To my knowledge, the answer to the first question is "no". The answer to the second question is probably "yes", based on the following reported observations on plasma levels of tea catechins in humans:

(i) After oral consumption, a large fraction of tea flavonoids is eliminated into the feces and bile. Only a fraction of the consumed flavonoids is absorbed into the circulation from the small intestine. The low bioavailability of the flavonoids is probably due to their high molecular weights and the ability of the phenolic OH groups to form large hydration shells [125, 126]. After absorption, EGCG is largely present in plasma in the free form, whereas EGC and EC are largely present in the conjugated form. Over 90% of the total absorbed EGC and EC is excreted in the urine, mostly in the conjugated form [127]. The evidence also suggests that some of the plasma catechins find their way to other tissues [128]. Catechins administered orally to pregnant rats were present in the placentas and fetuses [129].

(ii) Multi-dose oral consumption of EGCG and a concentrated green tea extract (Polyphenon E) by 40 healthy human volunteers at a daily dose of 800 mg ECGC (equivalent in EGCG content of 8–16 cups of green tea) for up to 4 wk was safe and well-tolerated [130–132]. Plasma levels of ECGC increased with amount consumed, reaching maximum values of 438 ng/mL. The proportion of free EGCG *versus* total (free and conjugated) remained unchanged after 1 month of daily tea consumption. Related studies with human volunteers [125, 133] report similar maximum plasma EGCG levels. Consumption of 1.5 mmol of three catechins (ECG, EGC, and EGCG) by ten human volunteers resulted in a peak plasma catechin level of 6.7 μmol/L [134]. Addition of milk to black tea did not significantly affect plasma catechin levels of 12 human volunteers [135].

(iii) In contrast, drinking of a tea preparation equivalent to 2–3 cups of tea resulted in saliva catechin levels two orders of magnitude greater than peak plasma levels [136]. The saliva concentrations of EGC reached maximum levels of 43.9 μg/mL, and of EGCG, 22 μg/mL. Holding the tea solutions in the mouth without swallowing resulted in even higher saliva catechin levels. The authors state that these and related observations imply that absorbed catechins are secreted from the saliva glands into the oral cavity.

These cited studies suggest that although the bioavailability of flavonoids is low, multiple consumption of EGCG as well as of teas resulted in significant accumulation of catechins in most of the body organs with relatively high peak plasma levels [132, 137, 138]. Because micromolar levels of tea compounds can inactivate pathogenic bacteria,

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Table 1. Inhibitory activities of tea catechins, theaflavins, and teas against pathogenic and spoilage bacteria, protein toxins, viruses, and fungi listed alphabetically

Organism	Adverse effects	Inhibitors	References
Bacteria			
Ba. anthracis	Anthrax	EGCG	[84]
Ba. cereus	Food poisoning; emesis	Catechins, theaflavins, tea	[3, 23]
Ba. subtilis	Food poisoning	Teas	[22]
Ca. jejuni	Food poisoning; diarrhea	Tea	[24, 33]
Cl. difficile	Severe diarrhea	Tea phenolics	[153]
Cl. tetani	Tetanus	Thearubigin	[88]
Corynebacterium spp.	Infection	Tea extracts	[33]
E. coli spp.	Food poisoning; diarrhea	EGCG	[22, 31, 32, 36]
H. pylori	Ulcers; chronic gastritis	EGC, EGCG; tea	[40, 42, 43, 99, 142]
Haemophilus influenzae	Bacteremia; meningitis	Tea extract	[33]
Klebsiella spp.	Pneumonia	Tea	[33, 35]
Le. pneumophila	Legionellosis; pneumonia	EGCG	[45, 143, 144]
Li. monocytogenes	Food poisoning; listeriosis	Tea	[33, 38]
Mycob. tuberculosis	Tuberculosis	Catechins	[33, 46]
Mycoplasma spp.	Prostatitis	Catechins	[47, 70]
Bo. pertussis	Whooping cough	EGCG, TF3	[102]
Ocular bacteria	Eye infections	ECGC	[48]
Phytopathogens	Plant disease	Catechins, theaflavins	[79]
Porphyromonas gingivalis	Periodontal disease	EGCG	[58]
Pr. vulgaris	Wound infections	Teas	[22]
Ps. aeruginosa	Food spoilage	Tea extract	[33]
Ps. fluorescens	Food spoilage	Teas	[22]
Shigella spp.	Diarrhea	Tea	[35]
Salmonella spp.	Food poisoning; salmonellosis	Teas	[35, 59, 71]
Se. marcescens	Food spoilage	Tea extract	[33]
Spore-forming bacteria	Food poisoning	Catechins	[28]
St. aureus	Food poisoning; infection	ECGC, TF3, theasinensin, tea	[22, 34–36, 61–63, 65, 66, 68, 76]
Str. mutans	Dental caries	Green, black, oolong teas	[51, 52, 55–57]
V. cholerae	Cholera	EGC, EGCG	[35, 36, 92, 93, 145]
Yersinia enterocolitica	Diarrhea	Teas	[33]
Toxins			
Anti-hemolysin alpha-toxin	Infections	Catechins, theaflavins	[145, 146]
Anthrax LF	Anthrax	Catechins	[84]
Botulinum neurotoxin	Botulism	Black tea	[89, 105]
Bo. pertussis	Whooping cough	Catechins	[100, 102]
Cholera	Cholera	Catechins; theaflavins	[145–147]
Helicobacter VacA	Ulcers	Polyphenol	[99]
Snake venom	Acute toxicity	Black tea	[103]
Staphylococcus enterotoxin B	Dermatitis	Catechins	[104]
Tetanus neurotoxin	Neurotoxicity	Catechins	[88, 105]
Vero (Shiga)	Diarrhea	EGCG, GCG	[107, 108]
Viruses			
Adenovirus	Respiratory infections	Catechins; tea	[111]
Bacteriophages, antiviral	Infection	Teas	[112]
Bovine coronavirus	Gastroenteritis in cattle	EGCG	[113]
Rotavirus, human	Infant diarrhea	Tea	[148, 149]
Bovine rotavirus	Gastroenteritis in cattle	EGCG	[113]
Epstein-Barr	Infectious mononucleosis	EGCG	[114]
Herpes simplex	Orofacial infections	Catechins; procyanidin	[115, 116]
Human immunodeficiency	AIDS	Catechins, theaflavins	[118–120, 150]
Influenza	Influenza	Catechins	[121, 122, 151]
Fungi			
Can. albicans	Candidiasis	EGCG; tea	[69, 123, 124]
Trichophyton	Skin infection	Catechin; tea	[123]

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the human bioavailability data suggest that long-term consumptions of tea can result in the absorption and retention of sufficient amounts of flavonoids to exert beneficial antimicrobial effects directly in plasma and tissues or indirectly by modulating cell signaling pathways. Because tea consumption results in high saliva catechin levels, tea may be a promising remedy for infections of the oral cavity. There is a need to find out whether frequent consumption of both green and theaflavin-containing black teas can protect against infection and/or ameliorate the severity of infectious disease in humans. The bioavailability of individual theaflavins has thus far apparently not been evaluated in human studies.

12 Research needs

In addition to research needs mentioned earlier, future studies need to address the following food and medical aspects of the properties of tea flavonoids and teas.

(i) Determine whether the potent antibiotic activities of flavonoids and teas *in vitro* can be duplicated *in vivo*, especially in humans.

(ii) Determine a presumptive connection between green or black tea consumption and lower risk of infection to humans.

(iii) Define additive and/or synergistic activities of mixtures of flavonoids with other plant-derived antimicrobials such as potato glycoalkaloids [117] and nonalkaloids such a cinnamaldehyde [1]. Combinations of food ingredients that act synergistically will lessen amounts needed to design effective antimicrobial food formulations. They will be safer and will affect flavor and taste less compared to the use of individual compounds.

(iv) Evaluate effectiveness of tea flavonoids against antibiotic-resistant foodborne pathogens. Acquired resistance to medicinal antibiotics is an important problem in microbiology [139]. Antibiotic-resistance often arises from administration of subtherapeutic levels of antibiotics in animal feeds. Resistant microorganisms may be present in the animal waste, often contaminating groundwater, surface water, irrigation water, fruits, vegetables, and other edible plant tissues. They suffuse throughout the food chain and can enter the human intestinal tract after the produce or undercooked food is eaten. Postulated antimicrobial mechanisms for botanicals involve disruption of microbial cell membranes and chelation to essential trace elements such as zinc and iron that the bacteria need for growth [6]. These mechanisms differ from those postulated for some antibiotics such as penicillin which act by inhibiting the formation of terminal dipeptides into peptidoglycans (transpeptidation) [140]. These considerations suggest the need to develop new alternatives for standard antibiotics based on flavonoids and other plant compounds that can be effective against antibiotic-resistant bacteria.

(v) Determine effectiveness of tea flavonoids in various foods including fruits and fruit juices, vegetables and vegetable juices, milk and cheeses, and meat and poultry products. For example, previously we developed wine formulations containing plant essential oils and oil compounds effective against the foodborne pathogenic bacteria *E. coli* O157:H7 and *Sa. enterica* [9]. The question arises as to whether wine formulations containing antimicrobial tea flavonoids can be devised to enhance microbial food safety and human health. A second example is our recent finding that a high-catechin containing green tea extract prevented sporulation and growth of *Cl. perfringens* in ground meat and poultry products (Juneja, *et al*., submitted).

(vi) Develop methods to concurrently reduce both pathogen and carcinogenic heterocyclic amine levels in processed meat products [29, 30].

(vii) Develop flavonoid-containing antimicrobial films and coatings to protect foods against contamination by pathogens [8, 141].

(viii) Determine whether the flavonoid content of teas is related to (can predict) antibiotic activities [3].

(ix) Determine whether flavonoid metabolites (glucoronides, sulfates) formed after absorption into the circulation by animals and humans possess antimicrobial properties.

(x) Determine whether molecular modeling of flavonoid structure–cell membrane interactions can be used to predict antibiotic activities of structurally different flavonoids.

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