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Food Compounds Inhibit *Staphylococcus Aureus* Bacteria and the Toxicity of *Staphylococcus* Enterotoxin A (SEA) Associated with Atopic Dermatitis

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1. Introduction

Enterotoxigenic *S. aureus* is a major bacterial pathogen that develops multi-drug resistance to antibiotics (Pereira et al., 2009; Pu et al., 2011; Rhee & Woo, 2010). The enterotoxigenic bacteria and secreted toxins have been reported to cause clinical infections, and it has also been reported that they contaminate a broad variety of foods, including breaded chicken products (Pepe et al., 2006), canned mushrooms (Anderson et al., 1996), cheeses (Ertas et al., 2010; Ostyn et al., 2010; Rosengren et al., 2010), raw milk (Fusco et al., 2011; Heidinger et al., 2009), pork meat (Wallin-Carlquist et al., 2010), and other foods (Balaban & Rasooly, 2000) as well contaminating handles of shopping carts (Mizumachi et al., 2011) causing many foodborne illnesses in the United States each year (Shinefield & Ruff, 2009). Staphylococcal food poisoning is due to the absorption from the digestive tract into the circulation of the enterotoxins preformed in food. A toxin level of $<1 \mu\text{g}$ may induce symptoms of food poisoning. This level is reached with a bacterial population of $>10^5$ CFU/g food (Stewart, 2005).

S. aureus produces the virulent staphylococcal enterotoxin A (SEA), a single chain protein that consists of 233 amino acid residues with a molecular weight of 27,078 Da. It has been estimated that staphylococcal enterotoxin A (SEA) secreted by the bacteria is associated with 78% of staphylococcal outbreaks (Vernozy-Rozand et al., 2004). Heat used to eliminate the pathogenic bacteria may not eliminate toxins already formed (Margosch et al., 2005; Pepe et al., 2006).

Staphylococcus enterotoxins (SEs) exhibit two separate biological activities: they cause gastroenteritis in the gastrointestinal tract and they act as a superantigen on the immune system. Previous research has shown that emetic activities and superantigenic activities of SEs are related (Shinefield & Ruff, 2009; Stewart, 2005).

Because SEA is present in contaminated foods and exerts adverse effects on the gastrointestinal tract, there is a need to find food-compatible safe conditions to inactivate it. Efforts to inhibit the toxin or its release from *S. aureus* include the use of electrolyzed water (Suzuki et al., 2002), high pressure and heat (Margosch et al., 2005), radiation and pulsed electric fields (Walkling-Ribeiro et al., 2008), condensed tannins (Choi et al., 2007) and other plant extracts (Carlos et al., 2010; Ifesan & Voravuthlkunchai, 2009), peptides (Wang et al.,

2008), phenolic compounds (Rúa et al., 2010), licochalcone A (Qiu et al., 2010), essential oils (de Souza et al., 2010; Friedman et al., 2004a; Nuñez et al., 2007; Parsaeimehr et al., 2010; Qiu et al., 2011), and toxin-specific antibodies (Larkin et al., 2010).

The objective of our research effort is to discover food-compatible ways to inhibit or inactivate both the pathogen and the toxin. In this chapter we will briefly summarize our finding in these two areas that are also relevant to atopic dermatitis.

2. Experimental procedures

The following experimental methods were used to create the data shown in the Tables and Figures.

2.1 Antibacterial activities of plant compounds against antibiotic-resistant *S. aureus*

The following procedure was adapted from Friedman et al. (2004b). Stock suspensions, 1 mg/mL, of cinnamon oil, oregano oil, thyme oil, carvacrol, and perillaldehyde were prepared by vigorous vortexing in phosphate-saline buffer at pH 7. Dilutions from stock solutions were revortexed, to ensure even mixing, before being further diluted or added to the cell suspension in the microtiter plate. The two solids (β -resorcylic acid and dopamine) formed clear solutions in the buffer. Each test substance was prepared fresh before use. Individual wells of a microtiter plate (Becton Dickinson, Oxford, UK) were filled with 100 μ L of the test substance in phosphate-saline buffer and 50 μ L of the culture. Triplicate wells were used for each concentration, with phosphate-saline used in the blank. The plates were shaken and then incubated at 37 °C for 1 h. The number of organisms remaining viable was then determined by a Nutrient Agar Spread Plate Technique on a 10- μ L aliquot removed from each well. Plates were incubated overnight at 37 °C prior to counting.

2.2 Antibacterial activity of Phloxine B against *S. aureus*

The following procedure was adapted from Rasooly (2005). Bacteria (200 μ L) grown in BHI broth were removed during the logarithmic growth phase (OD of 0.3 at 600 nm) and spread over the surface of a BHI agar plate with a bent glass rod. Various concentrations of Phloxine B (10, 5, 2.5, 1.25 and 0.62 μ g) and 5 μ g of chloramphenicol (10 units) and 1.5 μ g of tetracycline (2.5 units) were added to the wells of these plates as antibiotic standards. Plates were then incubated for 16 h at 37 °C in the light and in the dark. The potency of Phloxine B was determined by measuring the diameter of the inhibition zone made compared to zone produced by the standard antibiotics.

2.3 Antibacterial activity of 4-hydroxytyrosol and Hidrox-12 against *S. aureus*

The procedure was adapted from Friedman et al. (2011). *S. aureus* bacteria were grown overnight at 37 °C under aerobic conditions maintained by shaking at 200 rpm in Lucia-Bertani agar (LB) medium. The effect of 4-hydroxytyrosol and Hidrox-12 on bacterial growth was studied by addition of a bacterial suspension ($\sim 10^4$ diluted from $\sim 10^8$ bacteria/mL) to various concentrations of 4-hydroxytyrosol (range: 0.71-0.022 mg/mL) and Hidrox-12 suspension (range: 1.29-0.040 mg/mL) in phosphate buffer pH 7.0. After 60 minutes of incubation at 37 °C, 200 rpm, each dose/bacterial suspension was diluted 1/10 and then 20 μ L of each dose/bacterial suspension were dropped at the top of a square LB plate with grids. The plates were tilted and the drops ran down the plate. The plates were

dried (~10-15 min) and then incubated overnight 37 °C. Each dose was sampled in quadruplicate with control values ~100 colony forming units (CFUs).

2.4 Prophylactic vaccination of mice with *S. aureus*

The following procedure was adapted from Balaban et al. (1998). RAP (regulatory RNAPIII activating protein, 10 µg) was injected with CFA (completer Freund's adjuvant) on first injection, followed with ICFA (incomplete Freund's adjuvant) on second and third injections subcutaneously into 4-week old immunocompetent hairless male mice on days 0, 7, and 21. Control mice were either injected with the adjuvant alone or not injected. Vaccinated and control mice were challenged on day 31 with 1.24×10^8 CFU of wild-type Smith diffuse strain (SD) of *S. aureus* subcutaneously together with 1 mg of Cytodex beads to induce local infection. The size of the lesion was measured daily. Fisher's exact probability test was used to compare proportions of mice developing lesions and mice developing RAP antibodies among the RAP vaccinated, CFA controls, and untreated control groups. Among animals that developed lesions after challenge with *S. aureus*, the size of the lesions was compared by single-factor analysis of variance. Post-hoc testing was done by Fisher's protected least significant difference.

2.5 Staphylococcus enterotoxin (SEA) activity assays

The procedure was adapted from Friedman et al. (2011). Spleen cells were placed in 96-well plates (1×10^6 /mL, 0.2 mL) in Russ-10 medium and treated with various concentrations of SEA following incubation at 37 °C in a 5% CO₂ incubator. After incubation at various time points, cell proliferation was measured by adding bromodeoxyuridine (5-bromo-2-deoxyuridine, BrdU)-labeled DNA to each well 4 h before fixation as described by the instructions of the manufacturer. Spectroscopic measurements were made at 620 and 450 nm. A second measure of inhibition activity of SEA activity was determined by an enzyme cleavage assay. Briefly, the glycyl-phenylalanyl-aminofluorocoumarin (GF-AFC) substrate (50 µL) was added to the wells. After mixing and incubation for 30 min at 37°C, this substrate enters intact cells. The live cell protease then cleaves GF-AFC, releasing AFC which generates a fluorescent signal. The resulting fluorescence was measured by a fluorescence plate reader (excitation at 355 nm and emission at 523 nm). We used 5 and 200 ng/mL of the toxin because at (a) < 5 ng/mL we did not detect cell proliferation induced by the toxin; and (b) at ~200 ng/mL proliferation of spleen cells reach a maximum. Results are expressed as representative data from triplicate wells from two different methods, BRDU and the enzyme cleavage GFA-AFC assays which work only with live cells.

3. Results and discussion

Here, we briefly describe our studies designed to demonstrate the potential of bioactive food ingredients to inhibit growth of *S. aureus* bacteria and to inactivate SEA produced by these bacteria. The results suggest that it may possible to reduce the toxic potential of these toxin-producing organisms with the aid of edible food ingredients.

3.1 Naturally occurring compounds inactivate antibiotic-resistant *Staphylococcus aureus*

Antibiotic resistant microorganisms often arise from the administration of sub-therapeutic levels of antibiotics in animal feeds. They are present in the animal waste, often

contaminating groundwater, surface water, irrigation water, fruits, and vegetables. They can then disseminate through the food chain and enter the human intestinal tract after the produce or undercooked meat is eaten (Davis & Lederberg, 2001; Walsh, 2003). We showed that the following natural substances have antibacterial activity against three resistant pathogens including *Staphylococcus aureus* (ATCC12715): cinnamon oil, oregano oil, thyme oil, carvacrol, (S)-perillaldehyde, 3,4-dihydroxybenzoic acid (β -resorcylic acid), and 3,4-dihydroxyphenethylamine (dopamine) (Friedman et al., 2004a). Exposure of pathogens to a dilution series of the test compounds revealed that oregano oil is the most active substance (Table 1). Activities of the test compounds were in the following approximate order: oregano oil > thyme oil \approx carvacrol > cinnamon oil > perillaldehyde > dopamine > β -resorcylic acid. The order of susceptibilities of the pathogens to inactivation is: *B. cereus* (vegetative) >> *S. aureus* \approx *E. coli* >> *B. cereus* (spores). Some of the test substances may be effective against antibiotic-resistant bacteria in foods and feeds and in hospital environments.

	Concentration in well ($\mu\text{g/ml}$)				
Compound	0 (control)	66.7	6.67	1.34	0.067
Oregano oil	4.6 ± 0.08^1	<1.5	3.4 ± 0.12^c	4.6 ± 0.07^{ab}	4.6 ± 0.12^b
Thyme oil	4.6 ± 0.04	<1.5	4.5 ± 0.04^b	4.6 ± 0.09^b	4.6 ± 0.05^b
Cinnamon oil	4.6 ± 0.12	2.6 ± 0.58	4.6 ± 0.02^b	4.6 ± 0.07^b	4.7 ± 0.11^{ab}
Carvacrol	4.9 ± 0.12	0 ± 0	4.8 ± 0.21^a	4.8 ± 0.04^a	4.8 ± 0.12^a
Perillaldehyde	4.7 ± 0.05	4.1 ± 0.1	4.6 ± 0.11^b	4.6 ± 0.12^b	4.8 ± 0.02^a
	Concentration in well ($\mu\text{g/ml}$)				
	0 (control)	3330	670	330	67
Dopamine	4.6 ± 0.07	3.2 ± 0.33	4.5 ± 0.03	4.5 ± 0.11	4.6 ± 0.12
	Concentration in well ($\mu\text{g/ml}$)				
	Control	6000	5330	4670	3330
β -Resorcylic acid	4.8 ± 0.06	<1.5	<1.5	<1.5	4.6 ± 0.03

Table 1. Antibacterial activities of three plant essential oils (oregano, thyme, cinnamon), two essential oil compounds (carvacrol, perillaldehyde), dopamine, and the phenolic compound β -resorcylic acid) against antibiotic-resistant *S. aureus* (log CFU/ml; average \pm SD, n=3). CFU = colony-forming-units or bacterial counts. Superscript letters not in common are not significantly different (p<0.05). Adapted from (Friedman et al. 2004a).

3.2 The food dye Phloxine B inactivates *S. aureus*

The dye Phloxine B has been approved by the Food and Drug Administration (FDA) for human consumption. It is used in food, drugs, and cosmetics. We found that Phloxine B exhibits strong antimicrobial activities against several pathogenic bacteria including *S. aureus* (Rasooly, 2005). The diffusion assay we used to determine bactericidal effects shows that the activity of Phloxine B is similar to that of the medicinal antibiotics chloramphenicol and tetracycline (Figure 1). Figure 2 shows the dose-dependence of the inactivation in the range 25 to 100 $\mu\text{g/ml}$. A postulated mechanism of antimicrobial effects of the negatively charged dye is

described in some detail in the legend of Figure 3. It is likely that related charged polyaromatic compounds, including naturally occurring polyphenolic compounds, may operate by similar mechanisms. The results suggest that Phloxine B has the potential to be used as an antimicrobial agent against *S. aureus* in pathogens and in veterinary and human medicine and against other pathogenic bacteria. Its possible use against atopic dermatitis in human patients merits study.

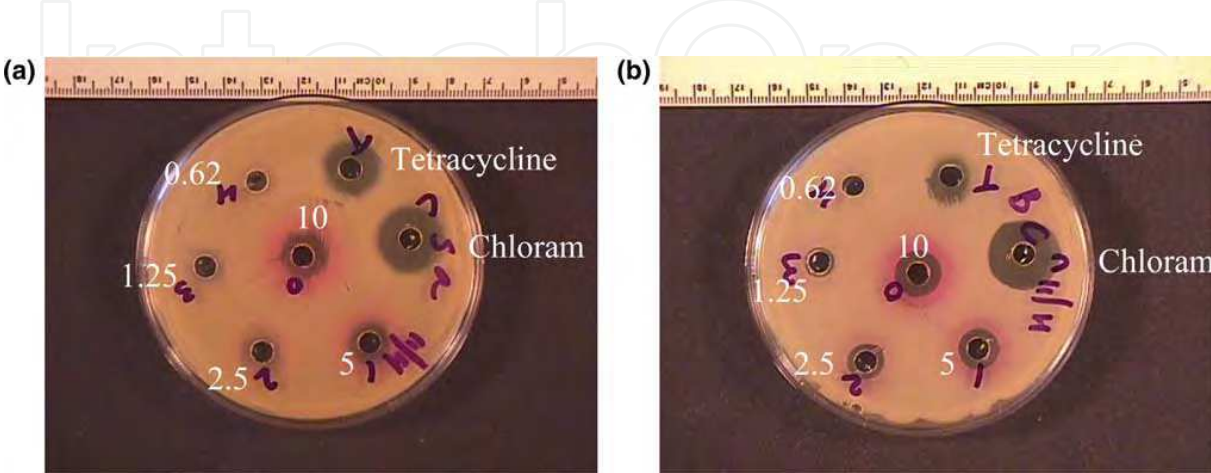


Fig. 1. Antibiotic potency of Phloxine B was determined by agar diffusion assay. Various concentrations of Phloxine B (10, 5, 2.5, 1.25, 0.62 µg) and 5 µg of chloramphenicol (10 units) and 1.5 µg tetracycline (2.5 units) were added to agar plates seeded with *S. aureus* (a), or *B. cereus* (b). Following incubation, the plates were examined for bacterial growth inhibition. Asdapted from (Rasooly, 2005).

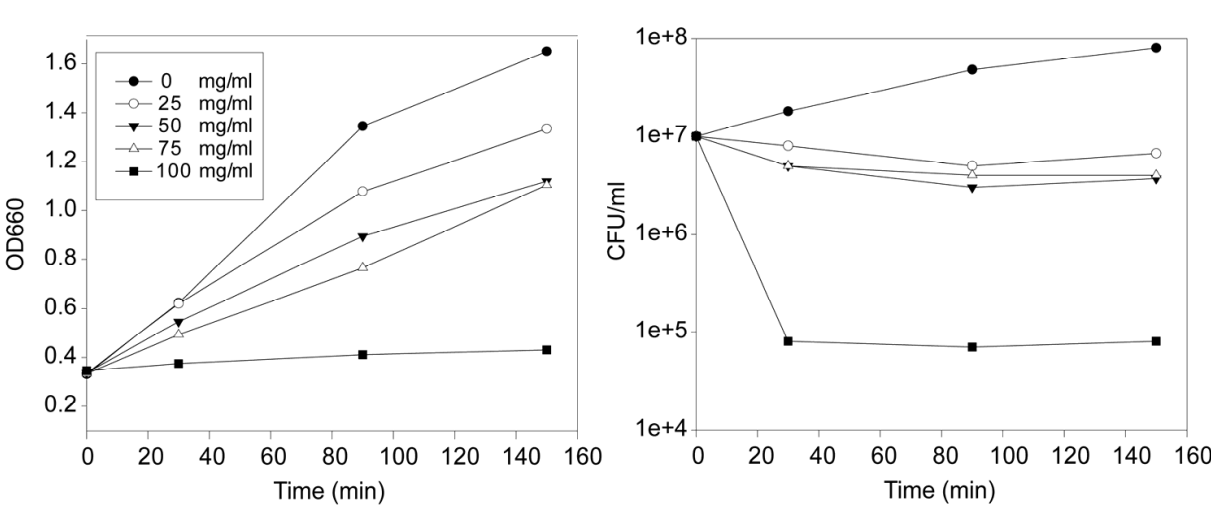


Fig. 2. Dose and time effect of Phloxine B (D&C Red#28) on *S. aureus*. Mid-log phase culture of *S. aureus* incubated with various concentrations of the dye. Turbidity of the media and bacteria viable cell count was measured at 20-minute intervals. Adapted from (Rasooly, 2005).

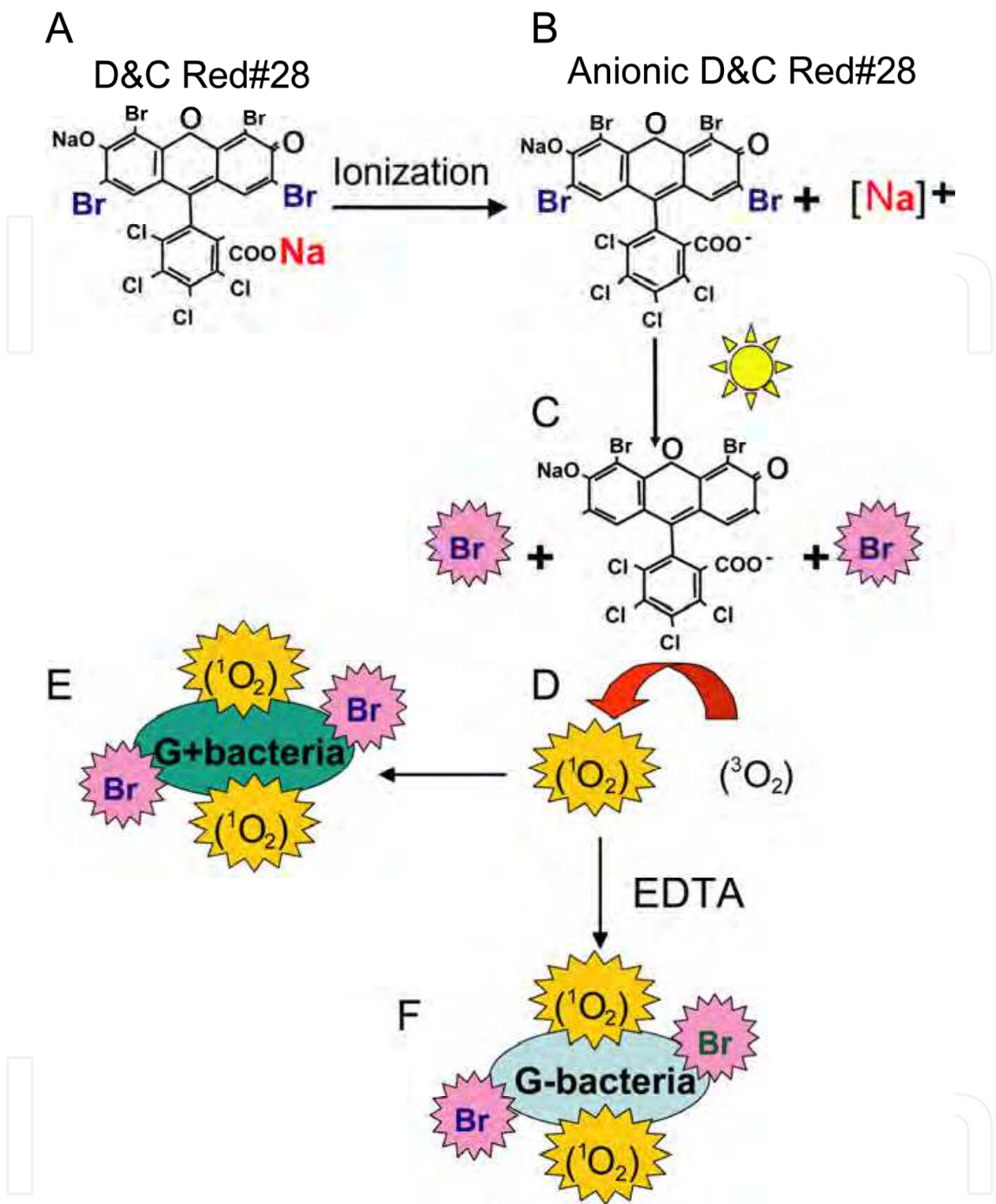


Fig. 3. The proposed mechanism of antimicrobial effects of the dye Phloxine B. Phloxine B (a) is ionized in water and (b) becomes negatively charged. The negatively charged anion (b) has a strong affinity for the positively charged cellular components such as proteins. Upon illumination (c), the photosensitized Phloxine B gains the energy associated with this light causing debromination and formation of free radicals and singlet oxygen (¹O₂) (d), which reacts with bacterial biomolecules (e), leading to cell death of the Gram-positive bacteria. The Gram-negative bacteria (f), which have highly negatively charged cell surfaces, repel the Phloxine B. Therefore, there is no binding and no antimicrobial effect between the dye and the Gram-negative bacteria. In the presence of EDTA, cell permeability increases, enabling dye penetration, leading to cell death. Adapted from (Rasooly, 2005).

3.3 Inhibition of SEA Release from *S. aureus* via autoinduction of virulence as a target for vaccine and therapy

With the increase in antibiotic resistance among staphylococci, there is an urgent need to develop vaccines to control bacterial infections. Although there are currently several vaccines at different stages of clinical development (Broughan et al., 2011), it seems that the development of a practical vaccine for which immunological responses can be monitored, thus enabling the prediction of its effectiveness in humans, is quite a challenging objective, not yet realized.

We believe that following novel approach we proposed merits further study (Balaban et al., 1998). The suggested approach is to interfere directly with bacterial virulence by interfering with transduction that leads to the production of toxins. This approach offers the possibility of transforming a toxin-producing pathogen to a non-pathogenic organism.

Antibiotic therapy against *S. aureus* is an important component of treatment for atopic dermatitis. However, the emergence of methicillin-resistant *S. aureus* (MRSA) presents new therapeutic challenges that suggest the need to develop new antimicrobial drugs and vaccines as an important objective. As mentioned earlier, the pathogenic effects of *S. aureus* are largely due to the production of bacterial toxins, especially SEA, which is regulated by the RNA molecule, RNAIII. The *S. aureus* protein called RAP activates RNAIII, and a peptide called RIP produced by non-pathogenic bacteria inhibits RNAIII. We discovered that mice vaccinated with RAP or treated with purified or synthetic RIP were protected against pathologic effects induced by *S. aureus*. (Table 2; Figure 4).

Treatment group	No. of mice	No lesions		Lesions		Death	
		<i>n</i>	(%)	<i>N</i>	Mean size (mm ²)	<i>n</i>	(%)
Vaccination with RAP as an antigen							
RAP	24	17	(71)	6	96	1	(4)
RAP*	9	7	(78)	2	84	0	(0)
CFA	10	3	(30)	5	177	2	(20)
Untreated	12	0	(0)	9	370	3	(25)
RIP suppression of 8.5 × 10 ⁷ SD							
SD+RIP	4	3	(75)	1	33	0	(0)
SD+saline	4	1	(25)	3	39	0	(0)
RIP suppression of 1.4 × 10 ⁸ SD							
SD+RIP	8	4	(50)	4	45	0	(0)
SD+saline	6	0	(0)	6	100	0	(0)
RIP and Pep suppression of 1.4 × 10 ⁹ SD							
SD+RIP	10	3	(30)	3	39	4	(40)
SD+saline	10	2	(20)	6	160	2	(20)
SD+Pep	10	9	(90)	1	56	0	(0)
SD+DMSO	9	2	(20)	4	128	3	(22)

Table 2. Vaccination or suppression of *S. aureus* SD infections. Adapted from (Balaban et al., 1998).

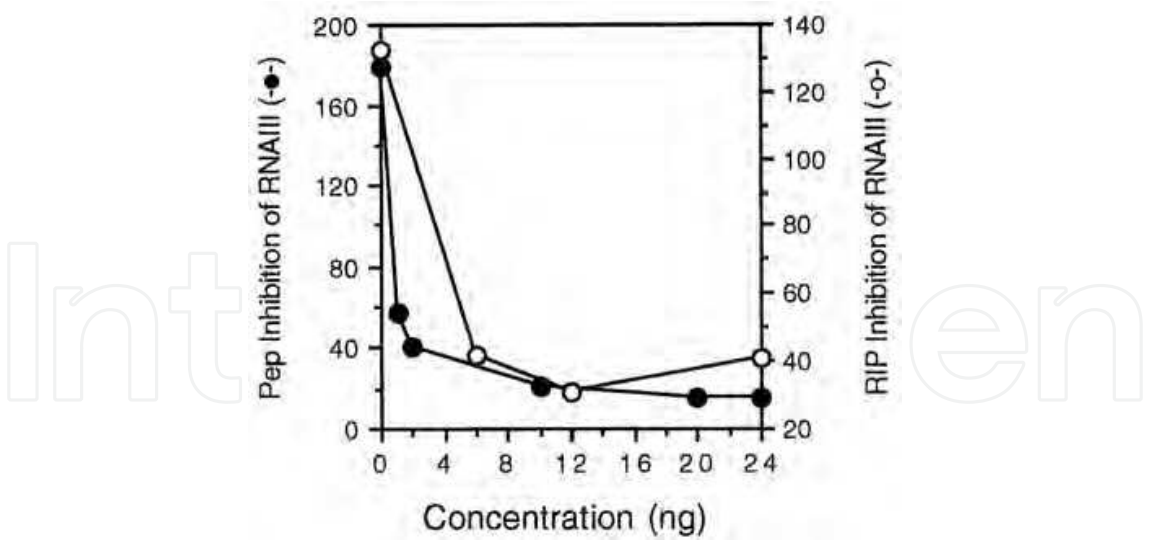


Fig. 4. Inhibition of RNAIII by native and synthetic RIP. Increasing amounts of RIP, which was purified on a C18 column or increasing amounts of synthetic peptide (Pep) were added to early exponential wild-type *S. aureus* and tested for the ability to inhibit RNAIII. Density of RNAIII is shown. $p<0.05$. Adapted from (Balaban et al., 1998).

Experimentally, we found that when we used increased inoculums of bacteria (1.4×10^8 cells per injection), four of eight animals were protected, and the remaining four developed a lesion that was 55% smaller than in control animals (Table 2). All the control animals (seven out of seven) challenged with SD and saline developed a lesion. When more bacteria were used (1.4×10^9), the synthetic RIP (0.5 mg of Pep) protected animals – 90% (9 out of 10) of the animals showed no sign of disease (Table 2). These results suggest that the ratio between RIP and the bacteria is critical and helps determine the success of the host's immune response to eliminate the bacteria.

These observations suggest that targeting autoinducers of virulence or the signal transduction they activate may, therefore, be a unique and useful approach in preventing pathogenesis of toxin-producing *S. aureus* and possibly other toxin-producing pathogenic bacteria.

3.4 Apple polyphenols inhibit T-helper cell proliferation and cytokine production in spleen cells from C57BL/6 female mice

As mentioned earlier, *S. aureus* is a major bacterial pathogen that causes clinical infection and food-borne illnesses (Dinges et al., 2000). This bacterium produces a group of twenty-one known enterotoxins (SEs) that have two separate biological activities: they cause gastroenteritis in the gastrointestinal tract and act as a superantigen on the immune system. Functional enterotoxins bind to the alpha-helical regions of the major histocompatibility complex (MHC) class II molecules outside the peptide-binding groove of the antigen presenting cells (APC), and also to the variable region (V β) on T-cell receptors. The toxin then forms a bridge between T cells and APCs. This event then initiates the proliferation of a large number (~20%) of T cells that induce the release of cytokines. At high concentrations, cytokines are involved in the etiology (causes) of several known human and animal diseases. These include atopic dermatitis and rheumatoid arthritis in humans (Lin et al., 2011; Macías et al., 2011; Yeung et al., 2011).

Atopic dermatitis (eczema) is an inflammatory skin disease that affects 10-20% of children and 1-3% of adults (1-3). Antibiotics that suppress colonization of *S. aureus* are reported to mitigate the severity of atopic dermatitis disease. Most strains of *S. aureus* isolated from atopic skin lesions produce exotoxins with superantigen properties (Leung et al., 1993). It has been reported that staphylococcal superantigens can induce skin inflammation by several different mechanisms (Taskapan & Kumar, 2000). The reason we selected the superantigen for this study is that it has been demonstrated to be an aggravating factor in atopic dermatitis, and because SEA is a representative antigen. It has been previously reported that consumption of apple condensed tannins from unripe apples improve the symptoms associated with atopic dermatitis (Kojima et al., 2000). The mechanism of this improvement is largely unknown. The main objective of our study was therefore to determine whether the beneficial effect of apple polyphenols is due to binding and inhibition of the superantigen and/or to inhibition of cell proliferation. In the present study, we evaluated the ability of one commercial and two freshly prepared apple juices and of a commercial apple polyphenol preparation (Apple Poly®) to inhibit the biological activity of SEA. The results are depicted in Figures 5-9. Dilutions of freshly prepared apple juices and of Apple Poly® inhibited the biological activity of SEA without any significant cytotoxic effect on the spleen cells. Additional studies with antibody-coated immunomagnetic beads bearing specific antibodies against the toxin revealed that SEA added to apple juice appears to be largely irreversibly bound to the juice constituents (Fig. 8). The results suggest that food-compatible and safe anti-toxin phenolic compounds can be used to inactivate SEA in vitro and possibly also in vivo, even after the induction of T-cell proliferation by long-term exposure to SEA. The significance of the results for microbial food safety and human health is discussed.

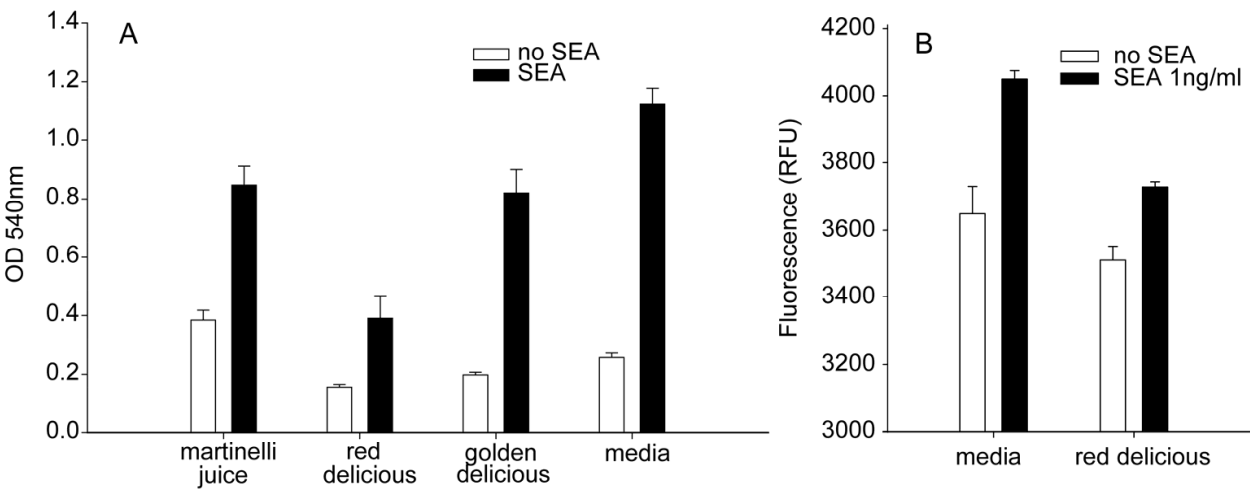


Fig. 5. Effect of apple juice on splenocyte proliferation. Apple juice and media used as a control with or without SEA (1 ng/mL) were incubated for 48 h with splenocyte cells followed by determining newly synthesized DNA (A) by cleavage of the peptide GF-AFC and (B) by use of live splenocytes. Error bars (n = 3) represent standard errors. Adapted from (Rasooly et al., 2010).

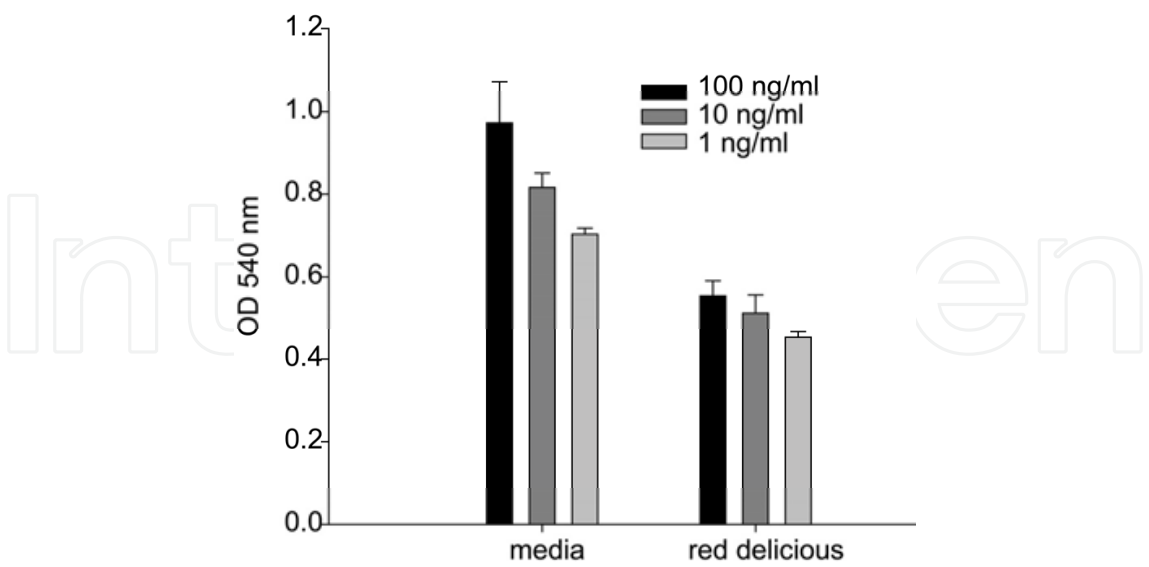


Fig. 6. Red Delicious apple juice inhibits high SEA concentrations. Three concentrations of SEA were incubated for 48 h with splenocyte cells followed by determining newly synthesized DNA. Error bars (n = 3) represent standard errors. Adapted from (Rasooly et al. 2010).

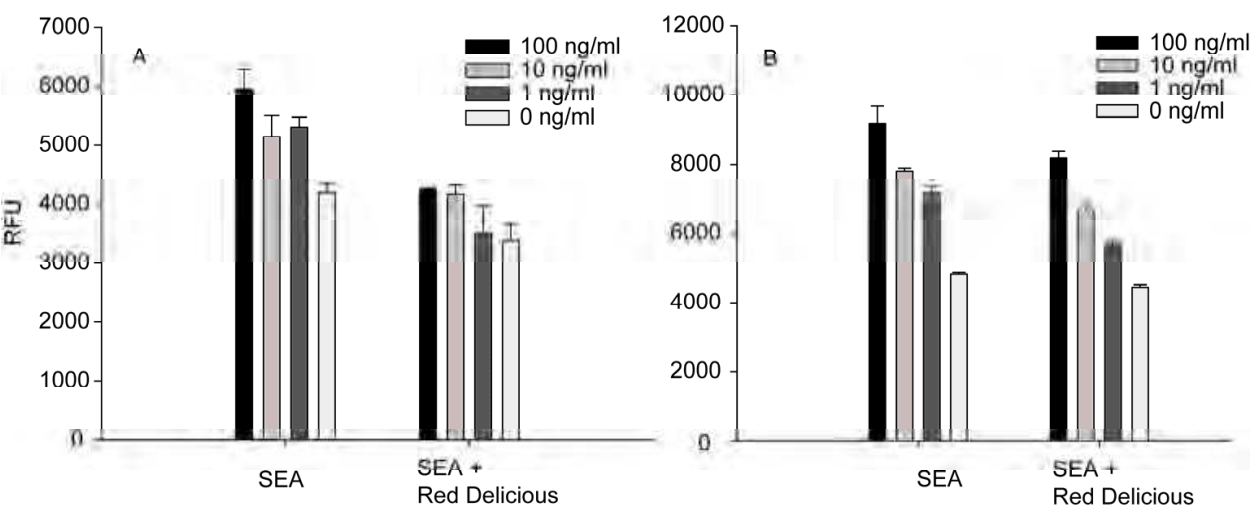


Fig. 7. Red Delicious apple juice reduces the activity of SEA after 24 or 48 h of incubation. Different amounts of SEA were added to the splenocytes, which were then incubated for 24 or 48 h. This was followed by the addition of Red Delicious apple juice and the determination of biological activity by cleavage of GF-AFC, produced by the live splenocyte cells after (A) 48 h or (B) 72 h. Error bars (n = 3) represent standard errors. Adapted from (Rasooly et al., 2010).

These observations suggest that Red Delicious juice has an inhibitory effect even after cell proliferation was initiated. We suggest that components of the juice disrupt the connection between antigen presenting cells (APCs) and T cells. Our results also imply that the mechanism by which consumed apple juice or apples may decrease the symptoms associated with atopic dermatitis is via inhibition of proliferation of T cells and the release of cytokines. The postulated mechanism that may govern the inhibition of the biological activity of SEA by apple compounds is visually illustrated in Figure 9.

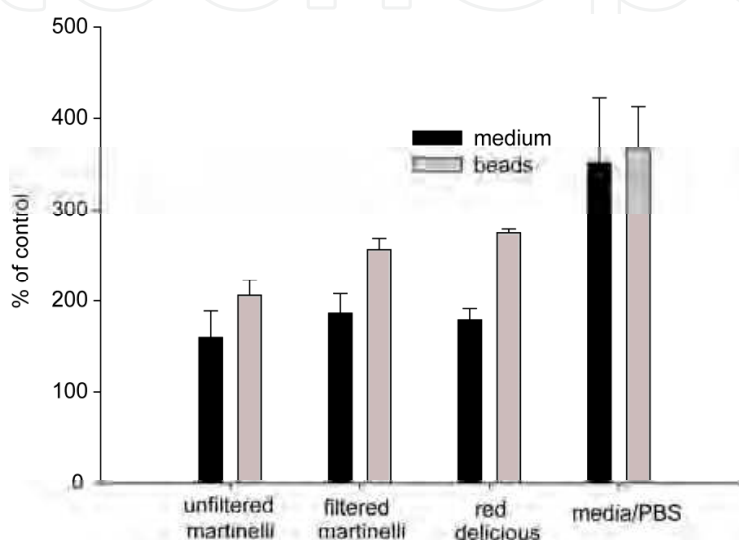


Fig. 8. Extraction and elution of SEA from apple juice treated with immunomagnetic beads. Apple juices were spiked with SEA (1 ng/mL) and incubated for 16 h with immunomagnetic beads. The toxin was dissociated from the beads and incubated with spleen cells. This was followed by the determination of newly synthesized DNA. Error bars (n = 3) represent standard errors. These results indicate that SEA added to apple juice appears to be largely irreversibly bound to the juice constituents. Adapted from (Rasooly et al. 2010).

The described findings suggest that apple juices and polyphenol-rich apple skin extracts have the potential to counteract adverse effects in animals and humans induced by SEA, and possibly also by the foodborne pathogen *S. aureus* that produces this virulent toxin. It would be of interest to find out whether or not the inhibited toxin in apple juice is reactivated in the digestive tracts of animals and humans and whether or not phenolic compounds present in other juices can concurrently inhibit the growth of *S. aureus* and other pathogens and the toxins produced by the pathogens.

In summary, our studies with apple juice and apples skin extracts demonstrated that apple polyphenols strongly inhibited superantigen-induced T-cell proliferation and cytokine production. The results also indicate that the low inhibitory action of freshly prepared Red Delicious apple juice is enhanced by added apple polyphenols. Further studies are needed to determine whether or not this combination may protect against animal and human diseases induced by high cytokine levels.

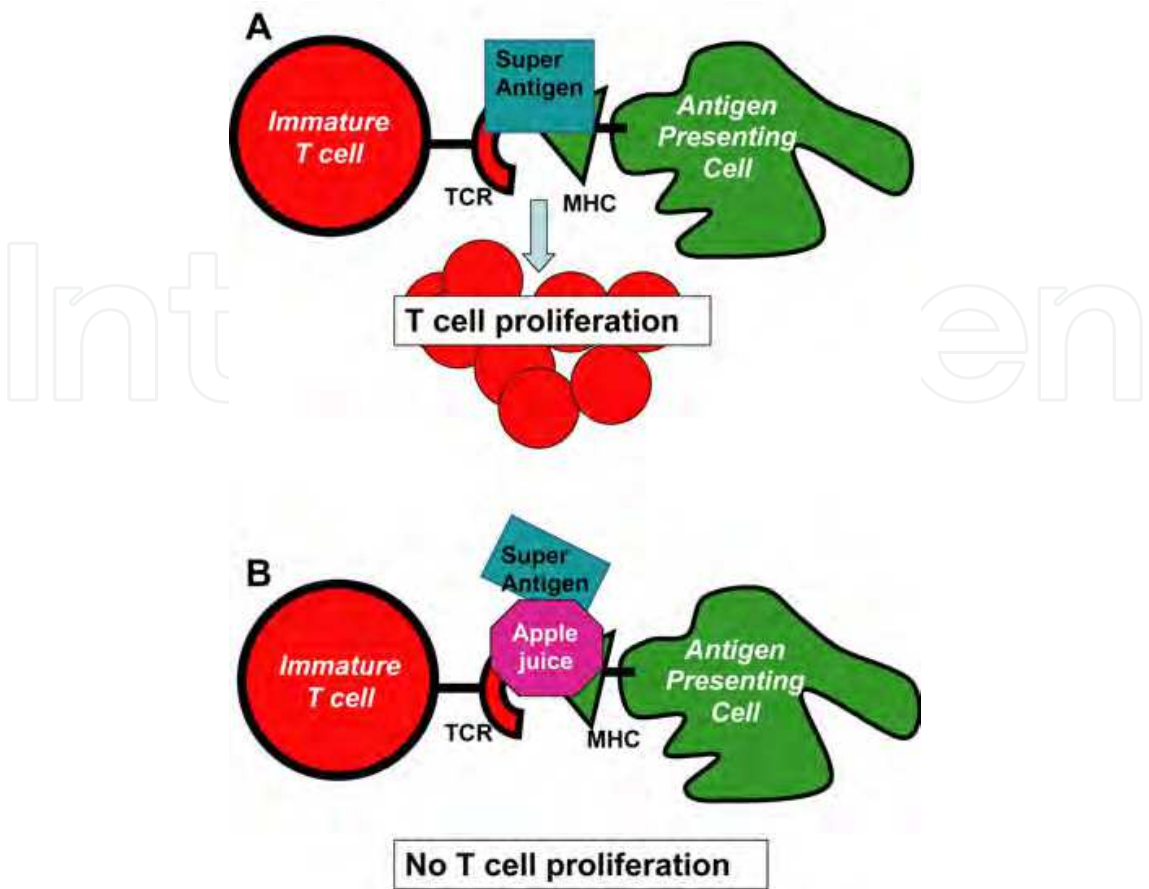


Fig. 9. A schematic representation of cellular events that lead to the inhibition of SEA-induced cell proliferation by apple juice. The individual steps in this scheme involve the (A) the formation of a bridge between APCs and T cells which results in induction of T-cell proliferation and (B) inhibition of T-cell proliferation by added pure apple juice that disrupts the connection between APCs and T cells. The net beneficial result of these events is the prevention of release and the consequent adverse effects induced by cytokines described in the Introduction. Abbreviations: MHC, major histocompatibility complex; TCR, T-cell receptor. Adapted from (Rasooly et al., 2010).

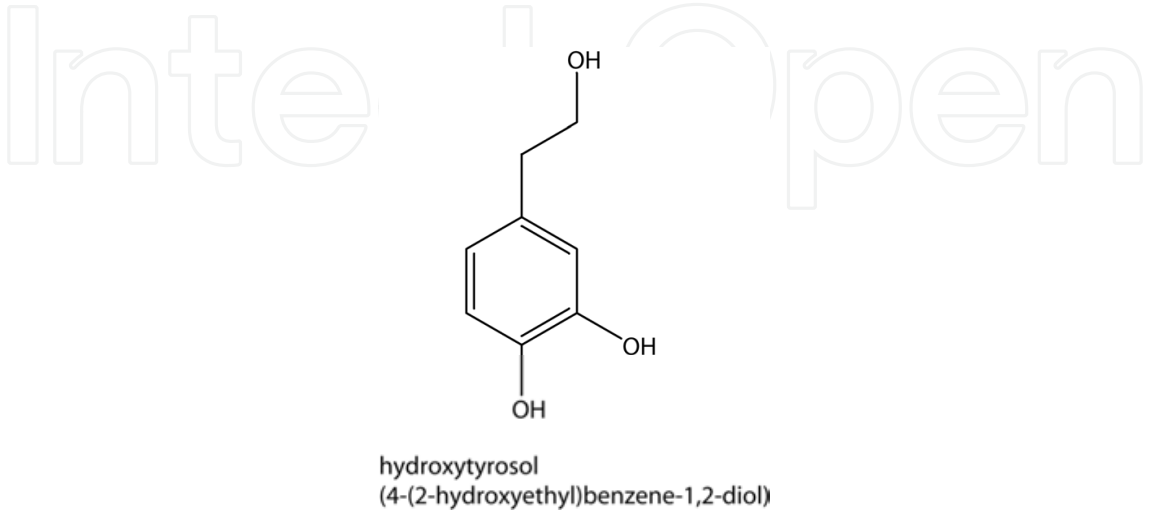


Fig. 10. Structure of the olive compound, hydroxytyrosol that inhibits *S. aureus* and SEA.

3.5 The olive compound 4-hydroxytyrosol inactivates both *S. aureus* and inhibits the biological activity of SEA

Our observations that dilutions of freshly prepared apple juices and of a commercial apple skin preparation inhibited the biological activity of SEA in a spleen cell assay suggested that other natural plant-derived compounds and plant extracts have the potential to inhibit the growth of foodborne pathogens and the toxicological effects of toxins produced by some pathogens (Rasooly et al., 2010). To further demonstrate this possibility, the objectives of another study were to determine whether the pure olive compound hydroxytyrosol (Figure 12) and a commercial olive powder named Hidrox-12 that contains hydroxytyrosol can inactivate *S. aureus* bacteria and inhibit the biological activity of SEA (Friedman et al., 2011). We found that olive ingredients also posses anti-*S. aureus* activity that reduced the counts of the bacteria in a dose-dependent manner. With hydroxytyrosol, the viable count of *S. aureus* bacteria after treatment at a concentration of 0.67 mg/ml for 60 min of contact, determined by plating on LB agar media, was decreased by 85%. We also found that dilutions of both test substances inactivated the pathogens.

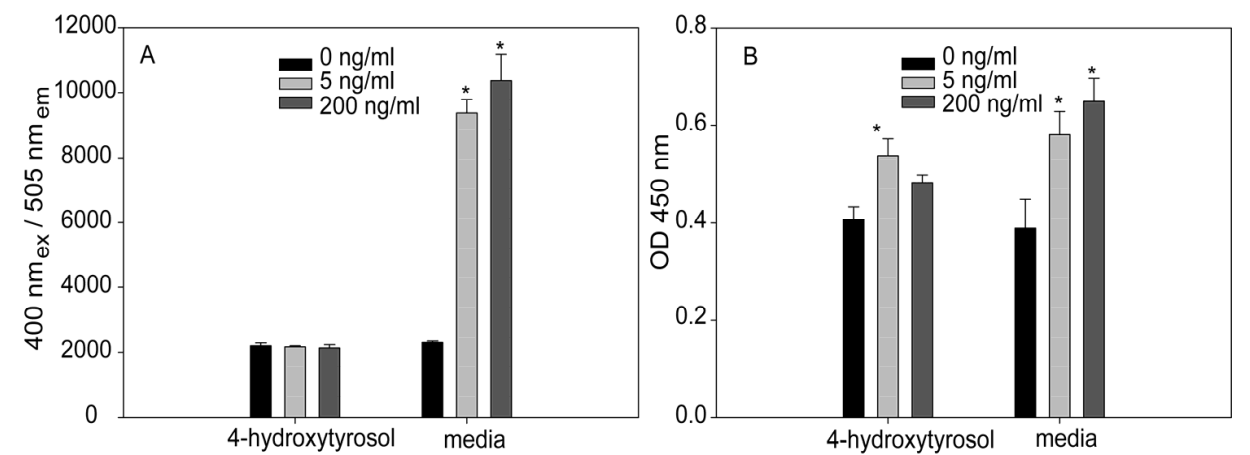


Fig. 11. Effect of hydroxytyrosol on splenocyte proliferation determined by two independent methods. Different concentrations of the toxin (0, 5, and 200 ng/mL) were exposed to hydroxytyrosol or the control (media) and were then incubated for 48 h with splenocyte cells followed by determining (A) GF-AFC cleavage by live cell protease (a measure of cellular activity) or (B) BrdU incorporation into newly synthesized DNA (a measure of cellular proliferation). Conditions: (A) GF-AFC substrate in intact cells is cleaved by live cell protease releasing the fluorescent AFC, quantified at an excitation wavelength of 355 nm and an emission wavelength of 523 nm. (B) BrdU (5-bromo-2-deoxyuridine)-labeled DNA was determined spectrophotometrically at absorbances of 620 nm and 450 nm. Error bars (n = 3) represent standard errors. Both assays show that hydroxytyrosol inhibited the biological activity of SEA. Error bars represent standard error (SEs), and an asterisk indicates significant differences (P<0.05) between treatments. Adapted from (Friedman et al., 2011).

Two independent cell assays (BrdU incorporation into newly synthesized DNA and glycyl-phenylalanyl-aminofluorocoumarin (GF-AFC) proteolysis) demonstrated that the olive compound also inhibits the biological activity of SEA. The described findings suggest that olive compounds have the potential to counteract adverse effects induced by SEA and by

the foodborne pathogen *S. aureus* that produces this virulent toxin. To our knowledge, this is the first report that demonstrated with the aid of bactericidal and cell assays that the edible olive compound hydroxytyrosol can inactivate both *S. aureus* bacteria and SEA. The results suggest that food-compatible and safe anti-toxin olive compounds merit further study designed to demonstrate their potential to treat atopic dermatitis. It would be of interest to extend these studies to the inactivation of other pathogens and toxins such as *E. coli* and Shiga toxin *in vitro* and in contaminated food such as meat, milk, and leafy greens.

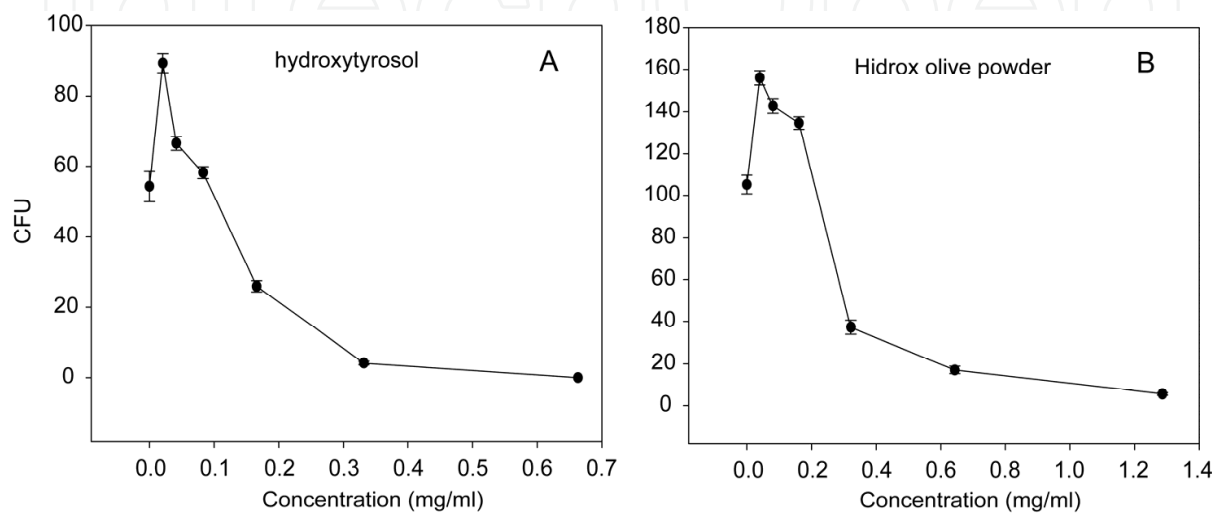


Fig. 12. (A) Antimicrobial activity of hydroxytyrosol against *S. aureus*. Conditions: bacteria were incubated with different concentrations of hydroxytyrosol. After incubation for 60 min, cells were plated and bacteria counted. (B) Antimicrobial activity of Hidrox-12 against *S. aureus*. Conditions: similar to those used for hydroxytyrosol. Error bars represent standard errors (n = 4). Both the pure olive compound and the olive extract inhibited the bacteria. The extent of inhibition by the extract was approximately equivalent to its content (12%) of hydroxytyrosol. Adapted from (Friedman et al. 2011).

4. Conclusion

S. aureus is a major bacterial pathogen that develops resistance to medical antibiotics. It has been reported to cause clinical infections and contamination of a broad variety of foods that may result in foodborne illness. These include canned mushroom, breaded chicken products, cheese, and raw milk as well on handles of shopping carts causing 185 000 cases of foodborne illnesses in the United States each year. *S. aureus* bacteria are present on the skin patients with atopic dermatitis. Many strains of *S. aureus* isolated from atopic skin lesions produce enterotoxins with superantigenic properties. *S. aureus* produces the virulent staphylococcal enterotoxin A, a single chain protein that consists of 233 amino acid residues. It has been estimated that the toxin that is secreted by the bacteria is associated with 78% of staphylococcal outbreaks. Our studies show that naturally occurring edible apple phenolic and olive compounds can both inactivate *S. aureus* bacteria and reduce the biological/toxicological properties of the toxin produced by these bacteria and that the food dye Phloxine B inhibits the release of SEA from the pathogens. Whether these novel approaches have therapeutic potential against atopic dermatitis and other diseases merits further study.

The described studies are part of a broader effort, the specific objective of which is to transform toxic proteins to nontoxic, digestible proteins in foods. For example, apple and other polyphenols present in numerous plant foods such as teas, sweet potatoes, and jujube fruits and seeds contain electron-rich aromatic structures and ionizable phenolic OH groups. These structures can in theory change the toxin via non-covalent binding to the toxin and/or by altering the distribution of ionic charges via H-bonding between OH groups and ionizable groups of the protein. We have no direct evidence for this theory, but note that in molecular simulation studies, we observed multiple hydrogen-bonding interactions between polyphenolic tea catechins and cell membranes that may result in anti-bactericidal effects due to disruption of the cell membranes followed by cell death (Sirk et al., 2009; Sirk et al., 2008; Sirk et al., 2011).

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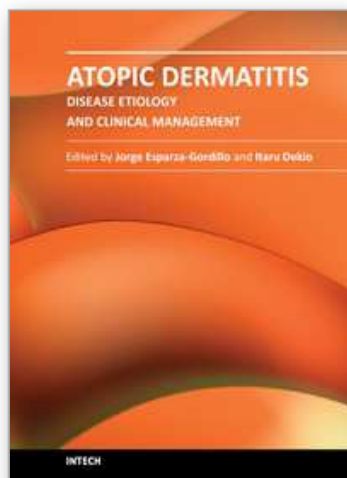
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Atopic Dermatitis is a common disease characterized by inflamed, itching and dry skin. This relapsing allergic disorder has complex etiology and shows a remarkably high clinical heterogeneity which complicates the diagnosis and clinical management. This book is divided into 4 sections. The first section (Disease Etiology) describes some of the physiological mechanisms underlying Atopic Dermatitis, including alterations in the immune system and the skin-barrier function. The important role of host-microorganism interactions on the pathophysiology of Atopic Dermatitis is discussed in the second section (Microorganisms in Atopic Dermatitis). An overview of the clinical diagnostic criteria and the disease management protocols commonly used is given in the third section (Diagnosis and Clinical Management). The last section (New Treatments) describes new therapeutic approaches that are not widely used but are currently being studied due to preliminary evidence showing a clinical benefit for Atopic Dermatitis.

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