Antibacterial activity of oregano tea and a commercial oregano water against *Escherichia coli* O157:H7, *Listeria monocytogenes* 4b, *Staphylococcus aureus* and *Yersinia enterocolitica* 03.

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Abstract

The amtimicrobial effect of oregano tea (1/10, w/v, in distilled water), commercial oregano water, and ½ oregano water (1/1, commercial oregano water/distilled water, v/v) against *Escherichia coli* O157:H7, *Listeria monocytogenes* 4b, *Staphylococcus aureus* and *Yersinia enterocolitica* 03 after direct contact at 20 ±2 °C for 1, 10, 30, and 60 min were comparatively evaluated. The Plate Count Agar was used for colony counts and the content of each treatment tube was enriched in Brain Heart Infusion broth after the 60 min direct contact period. All the four pathogens in oregano water and ½ oregano water decreased to uncountable level (1 CFU/ml) in one min. No growth has also occured in enrichments. Oregano tea was less effective than the oregano waters after 1 min, but counts of *E. coli* O157:H7, *L. monocytogenes* 4b, *S. aureus* and *Y. enterocolitica* 03 decrease to uncountable level after 60, 60, 10, and 10 min treatment periods, respectively. All the four test pathogens demonstrated grow ability in enrichment broth after a 60 min treatment period in oregano tea. *E. coli* O157:H7 appeared to be the most resistant pathogen followed by *L. monocytogenes* 4b, *S. aureus* and *Y. enterocolitica*, respectively. The use of water extracts or hydrosol of oregano for the decontamination or disinfection in food safety strategies should be evaluated in comparison with other natural antimicrobial spices.
Keywords: Antibacterial, oregano, pathogen.

Introduction

The benefical health effects of extracts from many types of plant that are used as seasoning agents in food and bewerages have been claimed for centuries (Friedman et al., 2002). Many hundreds of plants worldwide are also used in traditional medicine as treatments for bacterial infections (Cooke et al., 2000; Martin and Ernst, 2003; Okoli and Iroegbu, 2004). Scientists from divergent fields are investigating plants anew with an eye to their antimicrobial usefulness. A sense of urgency accompanies the search as the pace of species extinction continues. Laboratories of the world have found literally thousands of phytochemicals which have inhibitory on all types of microorganisms in vitro (Cowan, 1999).

The major origanum species, Oreganum onites (Turkish oregano), O. dictamnus, O. syriacum, O. minutiflorum, O. majorana grow wild in nearly all over the world. O. onites has a sharp, peppery flavor and a scent similar to thyme. Oregano spice is one of the flavorings and can be used fresh or dried in tea, salads and meat dishes. It contains acids, essential oils (EOs), minerals, tannins and vitamins. It is used as digestive, carminative, expectorant, antiinflamatory, antiseptic of the respiratory system, emenagouge, vulnerary, rheumatism, tonsillitis, cosmetics and cookery (Tucker and DeBaggio, 2000; Alma et al., 2003; Peñalver et al., 2004).

While food borne diseases remain an important public health threat worldwide, one of the most important food safety hazard is associated with the foods of animal origin. Raw and ready to eat food products are perceived to be responsible for significant amount of human illness because of the relatively high frequency of contamination with pathogens (Kessel et al., 2001; Zhao et al., 2001). The processes involved in raising and processing foods can introduce a variety of pathogenic microorganisms from several sources.

Antimicrobial agents are mostly synthetic chemicals and some agents are limited to use since they may cause adverse effects on public health and reluctance by consumers. Therefore, growing attention has been given to natural antimicrobials which are more readily accepted by consumers (Cowan, 1999). Antimicrobial effect of some extracts and essential oil concentrations of oregano on some microorganisms including food borne pathogens has been demonstrated (Skandamis and Nychas, 2001; Aridogan et al., 2002; Alma et al., 2003; Chorianopoulos et al., 2004; Dadalioglu and Evrendilek, 2004; Lin et al., 2004; Nostro et al., 2004). These studies has pointed out that the extract of these plants can be used for its antimicrobial property in many cases such as food safety and phytotherapy. But less studies has been conducted on the antimicrobial use of natural tea extract or hydrodistillation extract of the spices.

Tea is one of the most popular drink over the world, and some tea extracts has been found to have antimicrobial property (Diker et al., 1991; Yeo et al., 1995; Ahn et al., 1998: Kim et al., 2004). Many benefits of the herbal drinks are known well, antibacterial property of some of them is also being used in daily life (Cowan, 1999). Oregano water is sold commercially in Turkey for its systematic health benefits. It is also used to help the treatment of mouth and throat infections. The spice is also sold nearly all over the world in the form of herb tea and flavoring. Oregano has a good antioxidant capacity and also presents antimicrobial activity against food borne pathogens. These are all the characteristics of interest for the food industry because they may enhance the safety and stability of foods. We could not come accross to a study that is comparatively evaluated the antimicrobial activity of the oregano tea and commercial oregano water. The present work was undertaken to study the comparative antibacterial effect of oregano tea and commercial oregano water against *Escherichia coli* O157 (H7), *Listeria monocytogenes* 4b, *Staphylococcus aureus* and *Yersinia enterocolitica* O3 after direct contact application.

Materials and methods

Microorganisms

E. coli O157:H7 (strain no. 937) was kindly provided by Dr. Y. Ozbas (Univ. of Hacettepe, Ankara-Turkey) and *L. monocytogenes* 4b (strain no. SLCC 4013) from Munich Ludwig Maximillians Univ., and *Y. enterocolitica* O3 (KUEN846-23) from Istanbul Univ. Culture Collection Center- Istanbul, Turkey and *S. aureus* (NCTC 8325) from Refik Saydam Culture Collection Center- Ankara, Turkey. Each strain was maintained on tryptic soy agar with 0.6% yeast extract (Difco Laboratories, Detroit, Mich.) slants at 4^{o}C with monthly transfer. Each organism was transferred to tryptic soy broth with 0.6% yeast extract (Difco) and grown for 24 h at 30^{o}C before use.

Treatment solutions

Oregano tea: A 100 g commercial crumbled native oregano spice bought from a local retailer in Kars-Turkey added to 1 l boiled distilled water in a sterile erlenmayer flask, then the flask left for 30 min at 20±2 ^{o}C for making oregano tea 1/10 (w/v). After this time period, the content of the flask was heated at 95 ^{o}C for 1 min, and filtered throught a sterile cheesecloth to another sterile erlenmayer flask, then dispensed into sterile test tubes at 20 ml amounts. The tubes was cooled to room temperature and used in the experiments.

Oregano water and 1/2 oregano water: One liter commercial oregano (*Oreganum smyrnaeum*) water produced by Arifoglu Co., Turkey, (http://www.arifoglu.com) in glass botle was bought from a retailer in Kars-Turkey, and dispensed into separate sterile test tubes at 20 ml amounts. A 100 ml from the oregano water was also diluted in 100 ml of sterile distilled water for making 1/2 oregano water, then dispensed into separate sterile tubes at 20 ml amounts.

A 20 ml 0.1% peptone water and consequtive serial dilutiond mad in it was used for the determination of the initial inoculation level of each pathogen. A 10 ml sterile duble strength Brain Hearth Infussion broth (BHI, Oxoid) was used for enrichments.

Analyses

To evaluate the individual antibacterial activity of oregano tea and two different oregano waters against each of the four different test pathogens, 10 µl at each 18 h broth culture of one test pathogen was inoculated into 20 ml of the three different treatment tube, separately. The inoculated treatment tubes vortexed and left at room temperature (20 ±2 ^{o}C) during a 60 min direct contact time. After the 1, 10, 30 and 60 min time periods, a 2 ml from the each inoculated tube was neutralised in separate sterile tubes by adding 10% KOH solution. Then, 1 ml from the each tube was serially diluted with 9 ml of 0.1% sterile peptone water and viable counts were performed using a spread plate method on PCA plates. A 1 ml from each inoculated tube was also used for pour plating by using PCA in duplicate at the each analysis time. The plates were incubated at 30 ^{o}C for 48 h, and all the colonies on the plates were counted.

For the determination of presence of viable organisms in each treatment tubes after 60 min treatment period, 10 ml from each inoculated tube was added to 10 ml sterile duble strength brain heart infusion (BHI) broth and the tubes incubated 30 ^{o}C for 24 h. Then, a loopful form each enriched medium was streaked onto separate PCA plates. The plates were incubated at 30 ^{o}C for 48 h, and any growth on them demonstrated the viability of the test pathogens in the treatment tubes under countable level.

The experiment was repeated three times. Microbial counts were converted to $\log_{10}$ CFU/ml (countable limit was 1 CFU/ml). Descriptive statistics (means and standard deviations) were computed.

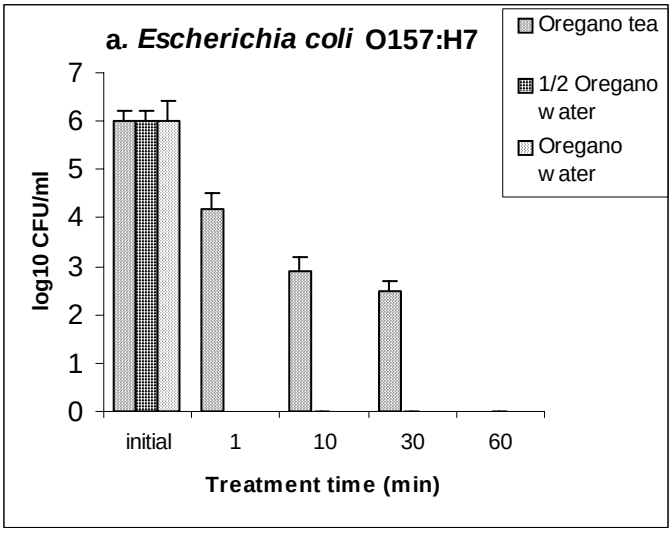
The pH of treatment solutions was measured with a pH meter equipped with an Orion-gel filled combination electrode (Fisher model 825MP) during the course.

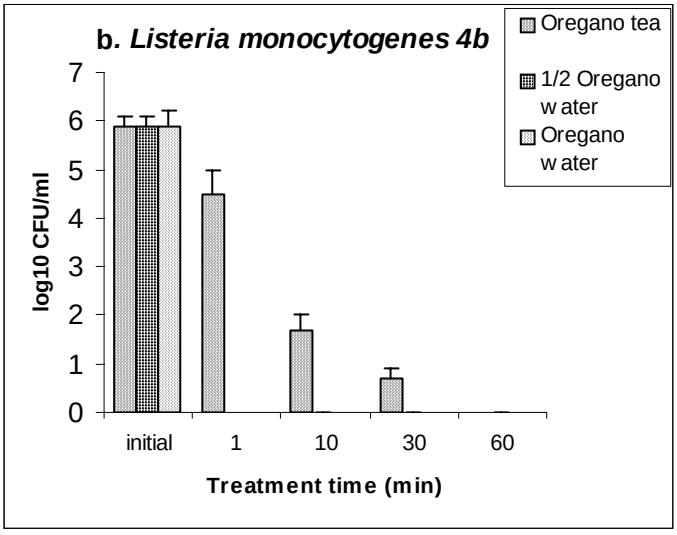
Results and discussion

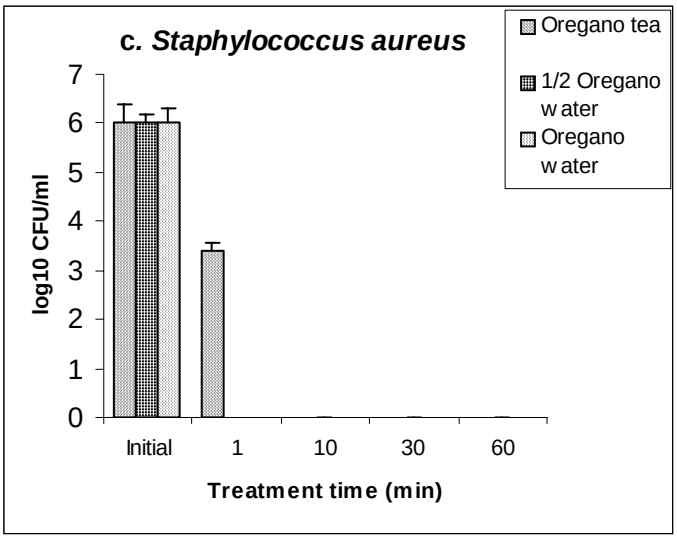
In pilot studies, we tested the two-fold serial dilutions of commercial oregano water and 1/10 oregano tea, but the best results were observed from the concentrations used in this study (data were not shown). The pH of the oregano tea, 1/2 oregano water and oregano water was 6.6, 4.2, and 4.0, respectively. A correaltion between acidity and antimicrobial activity of the treatment solutions were determined.

Commercial oregano water and 1/2 oregano water completely degrated all the four test pathogens

in 1 min. No growth were also determined from the enrichment of these treatment tubes. Oregano tea was also reduced the counts of the four test pathogens in 1 min. Nevertheless, complete inactivation time for each of the pathogen in oregano tea was different. After 60, 60, 10, and 10 min time periods, *E. coli* O157:H7 (Fig. 1a), *L. monocytogenes* 4b (Fig. 1b), *S. aureus* (Fig. 1c) and *Y. enterocolitica* (Fig. 1d) could not developed colonial growth on PCA plates, respectively. When a 10 ml from these test tubes was enriched in BHI broth after 60 min treatment period, all the four test pathogens demonstrated growth ability. Test pathogens were not completely be inhibited in oregano tea. Oregano water was more inhibitory on the test pathogens than the oregano tea. *E. coli* O157:H7 appeared to be the most resistant pathogen followed by *L. monocytogenes* 4b (Fig. 1b), *S. aureus* (Fig. 1c) and *Y. enterocolitica* (Fig. 1d), respectively.







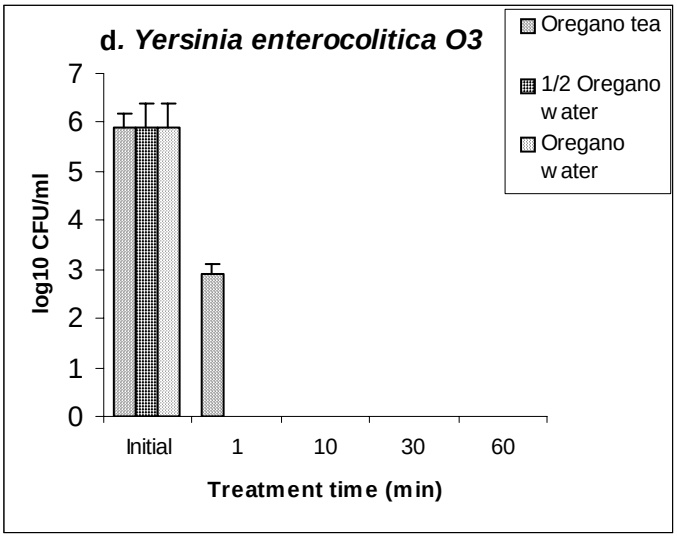


FIGURE 1. The amtimicrobial effect of oregano tea (1/10, w/v, in distilled water), commercial oregano water, and ½ oregano water (1/1, commercial oregano water/distilled water, v/v) against *Escherichia coli* O157:H7 (1a), *Listeria monocytogenes* 4b (1b), *Staphylococcus aureus*(1c) and *Yersinia enterocolitica* 03 (1d) after direct contact at 20 ±2 ^{o}C for 1, 10, 30, and 60 min. The Plate Count Agar was used for colony counts.

Preservatives used in the Agro-food industries may be of natural origin or obtained commercially. Because of the increasing interest of consumers in food products that contain only natural ingredients, studies on preservative molecules of natural origin, such as organic acids or peptides, have been reported in the past several years (Barreteau et al., 2004). A number of EOs and several of their individual components extracted from plants including oregano exhibit antibacterial activity against food borne pathogens in vitro and, to a lesser extent, in foods. Current informations about this subject are well documented by Burt (2004). Burt has noted in his reviev that if the active substances are to be added to foods in greater concentrations than is currently norrmal practice for flavourings, further safety studies may be necessary. Oregano EOs are has also well documented in the review by the outhor, their individual and combined antimicrobial activity against pathogenic and spoilage microorganisms is conspicuous in the review.

In this study, *E. coli* O157:H7 was found to be the most resistant organism (Fig.1), and that supports the many other researchers (Ahn et al., 2004; Hara-kudo et al., 2004; Kim et al., 2004; Peñalver et al., 2004; Taguri et al., 2004; Basri and Fan, 2005). It was also pointed out in previous studies that Gram-positive bacteria are more

susceptible to antimicrobial extracts of plants than Gram-negatives (Dorman and Deans, 2000; Digrak et al., 2001; Rhee et al., 2003; Burt, 2004). In this study, both of the oregano waters completely inhibited the growth of both Gram-positives and Gram-negatives. In oregano tea, the Gram-positive *L. monocytogenes* 4b was more resistant than the Gram-negative *Y. enterocolitica* O3. Since it was found to be the most resistant pathogen of the fours in this study, *E. coli* O157:H7 can be used in such experiments as an indicator pathogen.

Both commercial oregano water and 1/2 oregano water showed bactericidal effect against both Gram-negative and Gram-positive bacteria in 1 min. These results could indicate that commercial oregano water, even diluted in water at equal amount, has broader and stronger antimicrobial activity than the other plant extracts mentioned before (Stecchini et al., 1993; Roh et al., 1996; Hammer et al., 1999; Aridogan et al., 2002; Chorianopoulos et al., 2004; Kim and Fung, 2004). Kim and Fung (2004) has demonstrated the 5% arrowroot tea extract in BHI broth has antimicrobial activity, and it could be used as antimicrobial agent. In their study, population of all the Gram-positive and Gram-negative test pathogens including *E. coli* O157:H7, *L. monocytogenes* and *Y. enterocolitica* has increased at least 4 $\log_{10}$ CFU/ml in the oregano tea broth tubes incubated at 35 ^{o}C for 24 h. In this study, no growth has occured in the enrichment media after a 60 min direc contact period. Antimicrobial edible plants including oregano and arrowroot can comparatively study in such studies. Elgayar et al. (2001) has also found that oregano EOs was superior than that of some other spices, and it completely inhibited the selected food borne pathogens and spoilage microorganisms. The bactericidal concentration (625 µl/l) at 10, 20, and 37 ^{o}C of oregano EOs has been stated to irreversibly damaged *E. coli* O157:H7 within 1 min (Burt and Reinders, 2003). But in another study, satureja (savory) EOs has stated to posses remarkable bactericidal properties, which are clearly superior as compared to those Origanum and Thymus species EOs (Chorianopoulos et al., 2004). These results demonstrates that many studies have to be conducted the best source of the safe antimicrobials among plants.

It is known well that not only essential oils in medicinal plants and spices has antimicrobial effect, but also many other substances including phenolics, polyphenols, flavones, flavonoids, flavonols, tannins, coumarins, alcoloids, lectins and polypeptides, in combination with the EOs or separate in the water extract of the plant, have such activities (Cowan, 1999; Kirbag, 1999; Cutter, 2000; Akiyama et al., 2001; Mozaddedul Haque et al., 2002; Smith et al., 2003; Hara-kudo et al., 2004; Nas, 2004; web-I). Cowan (1999) has pointed out the two implications in his review: first, that most active components are not water soluble, and the second, that the most commonly used solvents may not demonstrate the greatest sensitivity in yielding antimicrobial chemicals on an initial screening. This disparity should be examined as the new antimicrobials intensifies. Basri and Fan (2005) has reported that the aquous and acetone extracts of galls of Quercus infectoria (Oak) displayed similarities in antmicrobial activity on the bacterial species and as such, it is the potentiallly source of antimicrobials. Okoli and Iroegbu (2004) has demonstrated that the antimicrobial effect of water and methanolic extracts of some plants was similar, while methanolic extracts of others was superior. Tannins are dissolved best in water than in methanol and ethanol (Pansera et al., 2004). Novaday, a specially cloned line of the herb oregano, called Umass oregano offered meat and poultry processors a consistent source of antimicrobial activity (web-II). Since it is a practical to prepare and cheap, hydrodistillation products of plants can be evaluated primarily for their antimicrobial properties rather than other extraction products. Water extracts of plants, if bactericidal, may be more appropriate to prepare and use for decontamination than other kinds of extracts.

The results of this study demonstrates that the water extract of oregano, which is practical to prepare, edible, safe, and possibly cheap than commerciallly available syntetic food antimicrobials and decontaminating agents can be an alternative source for food processors.

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