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Antibacterial activity of sumac extract, thyme water and lactic acid against Escherichia coli O157:H7, Listeria monocytogenes 4b, Staphylococcus aureus and Yersinia enterocolitica O3

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Summary

This study aimed to determine the antimicrobial activities of sumac (Rhus coriaria L.) water extract (8.0 %, wt/vol), thyme (Thymus vulgaris L.) water (commercial hydrosol) and ½ thyme water (1:1, commercial hydrosol / distilled water, vol/vol) in vitro in comparison with lactic acid (1.0 %, vol/vol), against the foodborne pathogenic bacteria Escherichia coli O157:H7, Listeria monocytogenes 4b, Staphylococcus aureus and Yersinia enterocolitica O3. The test microorganisms were inoculated to the treatment solution tubes. All the inoculated tubes were kept at 20 ± 2°C for 60 minutes. The numbers of the four test bacteria decreased to an uncountable level (<1 cfu/ml) in 1 min in the thyme water. The counts of all the pathogens, with the exception of L. monocytogenes 4b, were lower than the countable level after 1 min in the lactic acid. Both lactic acid and ½ thyme water reduced the test bacteria counts to the uncountable level in 10 min. In the sumac extract, the reduction time periods were 10 min for E. coli O157:H7, 30 min for Staph. aureus and 60 min for L. monocytogenes 4b and Y. enterocolitica O3. However, when enriched after treatment, E. coli O157:H7 and Staph. aureus were grown in lactic acid, S. aureus was grown in ½ thyme water and E. coli O157:H7, Staph. aureus, and L. monocytogenes 4b were grown in sumac extract. Thyme water had the strongest antibacterial activity against both the Gram negative and the Gram positive bacteria tested, followed by lactic acid, ½ thyme water and sumac extract.

Keywords: pathogen, E. coli, Listeria, Staphylococcus

Antimicrobial agents such as organic acids have been widely used to control the microbial growth of pathogenic bacteria in foods for several decades (10). It is clear that the surface treatment of carcasses by spraying them with lactic acid solution reduces the surface microbial counts, thus extending the shelf-life and providing food safety (15). In folk medicine, sumac is used in treatment of indigestion, anorexia, diarrhoea, haemorrhage and hyperglycaemia (17). Common thyme has a very long history of folk use for a wide range of ailments (1). Thyme water used in this study is a hydrosol commercially available in the local markets (Turkey). The aim of the study was to determine the antimicrobial activity of sumac water extract and thyme water in comparison with that of lactic acid, which a GRAS (Generally Recognized as Safe) compound, against the foodborne pathogenic bacteria *E. coli* O157:H7, *L. monocytogenes* 4b, *Staph. aureus*, and *Y. enterocolitica* O3.

Material and methods

Microorganisms. *E. coli* O157:H7 (strain no. 937) was kindly provided by Dr. Y. Ozbas (University of Hacettepe – Ankara, Turkey), *L. monocytogenes* 4b (strain no. SLCC 4013) was supplied by Munich Ludwig Maximillians University, *Y. enterocolitica* O3 (KUEN846-23) was obtained from Istanbul University Culture Collection Center – Istanbul, Turkey, and *Staph. aureus* (NCTC 8325) was purchased from the Refik Saydam Culture Collection Center – Ankara, Turkey.

Treatment solutions. Sumac was purchased from a spice factory in the form of dehydrated fruits. To prepare a water extract, the sumac was soaked in sterile distilled water (8%, wt/vol) in a sterile plastic bag for 12 h at 45°C. The content of the bag was then crushed externally, filtered through cheesecloth to a sterile Erlenmayer flask, and boiled gently for 2 min. on a plate heater equipped with a magnetic stirrer (Are2, VELP®, Italy); the extract was cooled to room temperature. Thyme (*Thymus vulgaris* L.) water (commercial hydrosol) was bought from a retailer in Kars – Turkey. To prepare ½ thyme water, equal volumes of commercial hydrosol and sterile distilled water were mixed in a sterile Erlenmayer flask. Lactic acid solution (1% vol/vol) was prepared in sterile distilled water by using % 85 lactic acid (Birkoo Corpora-

tion, Denver, CO.). All of the treatment solutions were dispensed separately to sterile test tubes, at 20 ml volumes. Tubes containing physiological saline inoculated with test microorganisms were used as controls.

Test methods. For the determination of any microbial growth, 5 ml of each treatment solution was inoculated to BHI (Brain Heart Infusion Broth, Oxoid), and incubated at 30°C for 18 h. Then, 100 µl of the enriched samples was spread over PCA (Plate Count Agar, Oxoid) plates and incubated at 30°C for 48 h. To evaluate the antibacterial activity of the treatment solutions against the test bacteria, the tubes containing 20 ml of treatment solution and the control tubes containing 20 ml of physiological saline were inoculated with 20 µl of overnight (18 h) broth culture of the test microorganisms and kept at 20 ± 2°C for 60 minutes. At 1st, 10, 30 and 60th min. of the treatment period, 2 ml of each inoculated tube content were transferred to sterile empty test tubes and neutralised, with the exception of the controls, using 10% KOH solution. One ml of each sample was pour plated using PCA. Appropriate serial dilutions of the remaining samples were prepared in PW (Pepton Water, Oxoid), and 100 µl of each dilution was spread over PCA plates in duplicate. The plates were then incubated for 48 h at 30°C. The colonies grown on the plates were enumerated and the counts were converted to $\log_{10}$ cfu/ml. For the determination of the presence of test strains in the treatment solutions at uncountable level (< 1 cfu/ml) after the 60 min. treatment period, 10 ml of each treated tube content was added to tubes containing 10 ml double strength BHI broth (prepared with Brain Heart Infusion Broth, Oxoid). The content of enrichment tubes was neutralised by 10% KOH, and incubated at 30°C for 24 h. One ml of each enriched sample was pour plated using PCA. The plates were incubated for 48 h at 30°C and observed for any bacterial growth. To confirm the concentration of the inocula, 20 µl of overnight (18 h) broth culture of the test microorganisms was also inoculated to tubes containing 9 ml PW (Pepton Water, Oxoid) in parallel to treatment solutions, and consecutive serial dilutions were prepared in PW. A hundred µl of each dilution was spread over PCA plates. The plates were then incubated for 48 h at 30°C and those including 30-300 bacterial colonies were used for estimating the inoculation level (cfu/ml) of the test strains. The pH of the treatment solutions was measured by a pH meter equipped with an Orion-gel filled combination electrode (Fisher model 825 MP). Three replications of the experiment were made.

Results and discussion

The pH of these treatment solutions were 4.7, 3.2, 4.8 and 3.6, respectively. The inoculation levels of the test bacteria in the treatment solutions were 5.4, 5.7, 5.5, and 5.9 log10 cfu/ml for *E. coli* O157:H7, *L. monocytogenes* 4b, *Staph. aureus*, and *Y. enterocolitica* O3, respectively. The test bacteria counts remained constant in the physiological saline tubes at the end of the 60 min. treatment period (data not shown). All four test strains were completely inhibited in 1 min. by the thyme water (fig. 1), and no growth was observed after enrichment. *L. monocytogenes* 4b appeared to be more resistant to lactic acid than the other test microorganisms. Whereas the numbers of *E. coli* O157:H7, *Staph. aureus*, and *Y. enterocolitica* O3 were decreased to under the countable level (1 cfu/ml) at the end of the 1 min. treatment period, *L. monocytogenes* 4b was reduced from 5.7 to 2.6 log10 cfu/ml. The count of this strain was reduced to below countable level (1 cfu/ml) at 10th min of the treatment (fig. 2). However, *E. coli* O157:H7 and *Staph. aureus* growth was observed when an enrichment procedure was applied after 60 min. lactic acid treatment (data not shown). *E. coli* O157:H7 was reduced from 5.4 to 3.9 log10 cfu/ml by the ½ thyme water in 1 min., *L. monocytogenes* 4b from 5.7 to 3.6 log10 cfu/ml, and *Staph. aureus* from 5.5 to 3.6 log10

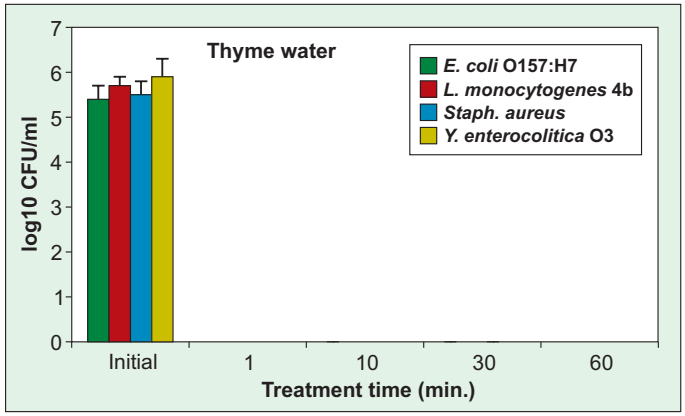

Fig. 1. The antimicrobial activity of thyme (*Thymus vulgaris* L.) water against *Escherichia coli* O157:H7, *Listeria monocytogenes* 4b, *Staphylococcus aureus* and *Yersinia enterocolitica* O3 at 20 ± 2°C at 1st, 10, 30 and 60th min of treatment period

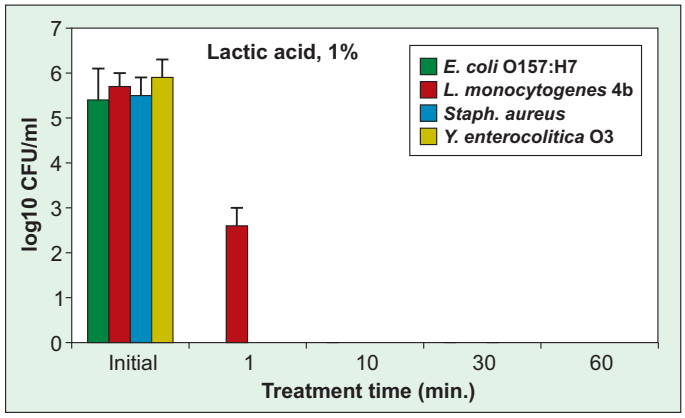

Fig. 2. The antimicrobial activity of 1% lactic acid against *Escherichia coli* O157:H7, *Listeria monocytogenes* 4b, *Staphylococcus aureus* and *Yersinia enterocolitica* 03 at 20 ± 2°C at 1st, 10, 30 and 60th min of treatment period

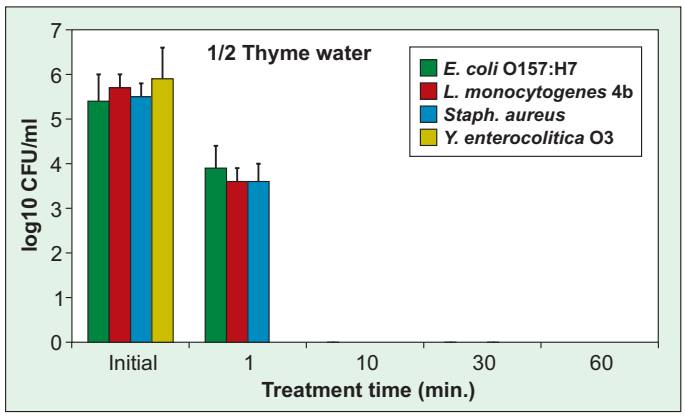

Fig. 3. The antimicrobial activity of ½ thyme water against *Escherichia coli* O157:H7, *Listeria monocytogenes* 4b, *Staphylococcus aureus* and *Yersinia enterocolitica* 03 at 20 ± 2°C at 1st, 10, 30 and 60th min of treatment period

cfu/ml. Although, all the test bacteria numbers were at the uncountable level after 10 min ½ thyme water treatment (fig. 3), *Staph. aureus* growth was observed after enrichment (data not shown). The counts of *E. coli* O157:H7, *L. monocytogenes* 4b, *Staph. aureus* and *Y. enterocolitica* O3 were reduced from 5.4 to 5.3, from 5.7 to 4.4, from 5.5 to 4.9 and from 5.9 to 5 $\log_{10}$ cfu/ml, respectively by sumac

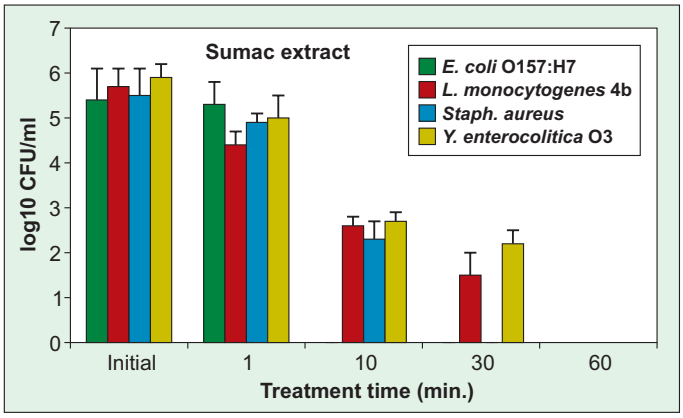

Fig. 4. The antimicrobial activity of sumac (*Rhus coriaria* L.) water extract against *Escherichia coli* O157:H7, *Listeria monocytogenes* 4b, *Staphylococcus aureus* and *Yersinia enterocolitica* 03 at 20 ± 2°C at 1st, 10, 30 and 60th min of treatment period

extract, in 1 min. After a 10 min. treatment *E. coli* O157:H7 was uncountable in the sumac extract tubes, while the levels of *L. monocytogenes* 4b, *Staph. aureus* and *Y. enterocolitica* O3 were 2.6, 2.3 and 2.7 $\log_{10}$ cfu/ml, respectively. At 30th min in the sumac extract tubes *Staph. aureus* was also reduced to below countable level, but the number of *L. monocytogenes* 4b was 1.5 and the *Y. enterocolitica* O3 count was 2.2 $\log_{10}$ cfu/ml. These two strains were decreased to uncountable level only after 60 min. (fig. 4). Nevertheless, *E. coli* O157:H7, *Staph. aureus*, and *L. monocytogenes* 4b growth was observed after enrichment (data not shown). The results indicated that thyme water had the highest level of antimicrobial activity against the test microorganisms followed by 1% lactic acid, ½ thyme water, and 8% sumac extract, respectively.

Although all the treatment solutions had an antimicrobial effect, thyme water demonstrated the strongest antibacterial activity against both Gram-negative and Gram-positive bacteria in this study. There was no correlation between the pH and antibacterial activity of the treatment solutions. The antimicrobial activity seemed to depend on the antimicrobial compound in the thyme water and sumac extract. In a study, Nasar-Abbas and Halkman (11) reported that the inhibitory action of sumac was not due only to its acid content but also to the presence of some antimicrobial substances. The acid content may have an inhibitory effect on the test organisms separately or it may have synergistic effects with the antimicrobial substances present in the plant.

Recently, a number of studies have examined the role of essential oils, extracts and decoctions of spices in inhibiting the growth of microbes (3, 5-8, 13). Cowan (2) reported that essential oils and many other substances, including phenolics, polyphenols, flavones, flavonoids, tannins, coumarins, alkaloids, lectins and polypeptides, in the water extract of plants have an antimicrobial effect. The hydrosol, the essential oil and the plant extract may all have different properties. Hydrosols are widely used in aromatherapy, however research into their antimicrobial activities *in vitro* is limited. Sagdic (16) indicated that two thyme (*Thymus vulgaris* L. and *Thymus serpyllum* L.) hydrosols at 50 and 75 ml/100 ml concentrations in nutrient broth had a bactericidal effect against *E. coli*, *E. coli* O157H:7, *Staph. aureus* and *Y. enterocolitica*, and that *Staph. aureus* was the most sensitive strain. In this study, all the test bacteria numbers were at the uncountable level after 10 min. ½ thyme water treatment. However *Staph. aureus*, unlike the others, was grown in enriched broth. In contrast to Sagdic (16), in the present study *Staph. aureus* was a more resistant strain to ½ thyme than the other bacteria tested. This may be due to the differences in the methods applied or strains used.

Sumac is rich in tannins, and the tannins are dissolved better in water than in methanol or ethanol (14). Tannins in plant extracts have been found to possess antibacterial activity (9). In a study conducted by Nascimento et al. (12), plant extracts containing tannins were found to be effective against microorganisms including *Staph. aureus* and *E. coli*. Nasar-Abbas and Halkman (11) reported that water extract of sumac had a bactericidal effect on all the organisms tested including *L. monocytogenes*, *E. coli* O157:H7, *E. coli* type I, *Salm. enteritidis*, and *Staph. aureus.* Digrak et al. (4) also found that the extracts of fruits of sumac was effective against *Staph. aureus*, and *L. monocytogenes*. The present study indicated that sumac water extract had antimicrobial activity against all the pathogenic bacteria tested. *E. coli* O157:H7, *Staph. aureus*, *L. monocytogenes* 4b and *Y. enterocolitica* O3 were inhibited in the sumac extract tubes at 10, 30, and 60th min. of treatment period, respectively. However, *E. coli* O157:H7, *Staph. aureus*, and *L. monocytogenes* 4b were grown in enrichment broth. *Y. enterocolitica* O3 was more sensitive to sumac extract than the other strains, while *L. monocytogenes* 4b was the most resistant strain. Our results were similar to those obtained by Nasar-Abbas and Halkman (11) and Digrak et al. (4).

Further studies are needed to evaluate the antimicrobial activity of these plant extracts in food models, slaughterhouses and food processing plants. Sumac extract and thyme water appeared to be potential sources of natural preservative, decontaminant and disinfectant.

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