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Antimicrobial effects of grape pulp, Bakhtiari savory, and lemon extracts on *Listeria monocytogenes* isolated from meat

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ABSTRACT

Listeria monocytogenes is a coccobacilli, gram-positive, non-spore forming bacteria. The distribution of these bacteria in nature is high, which is a human and animal pathogen and has different complications. The main transmission way for human infection is through the consumption of contaminated foods. This study is aimed to investigate the antimicrobial effects of Bakhtiari savory, grape pulp and lemon extracts against *Listeria monocytogenes*. The pulp extract of a kind of lemon and the aalcoholic extract of grape seed were extracted and the concentrations of 16 to 1000 mg/ml were prepared. The minced meat was infected with *Listeria monocytogenes*, and then the prepared concentrations of the extracts were injected into the meats carrying the bacteria. After 3, 7 and 10 days, a microbial culture was done from the meats and the colonies were counted. The study showed that the Bakhtiari savory extract and then the grape pulp has the greatest inhibitory effect both in vitro and in food. It was also shown that the Bakhtiari Savory could be effective as a preservative to increase meat durability and to prevent the growth of pathogens such as *Listeria monocytogenes*. According to the inhibitory effect of Bakhtiari Savory extract and the grape pulp on *Listeria monocytogenes*, the substances may be used for the treatment of diseases caused by these bacteria.

KEY WORDS: *LISTERIA MONOCYTOGENES*, MEAT, BAKHTIARI SAVORY, GRAPE PULP, LEMON

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INTRODUCTION

Listeria monocytogenes (*L. monocytogenes*) is a gram-positive, non-acid-fast and short rod shaped, opportunistic intracellular, Gram-positive, facultative anaerobic, nonspore-forming bacterium of the genus Listeria, which is widely spread in the environment, and can cause severe diseases including meningoencephalitis, septicemia, mastitis and abortion in humans and animals, primarily affecting pregnant, new-born, and immunocompromised individuals (Dehkordi et al. 2013, Reddy and Lawrence 2014).

According to previous epidemiological investigations, various types of foods and especially those with animal origins are the main reservoirs for virulent strains of *L. monocytogenes* (Dehkordi et al. 2013, Di Pinto et al. 2010, Gray et al. 2004, Tompkin 2002). The high ability of bacterium to resistance against environmental conditions such as temperature, pH, salt concentration and also dryness is the main reason for considerable role of the *L. monocytogenes* as an emerging food-borne pathogen (Mataragas et al. 2006, Zhang et al. 2012). Meat is raised as one of the most important reservoirs of the *L. monocytogenes* (Goh et al. 2012, Indrawattana et al. 2011, Siriken et al. 2013). Meat of animals prepares favorable conditions including optimum levels of nutritional materials, temperature, activated water (AW), pH and salt for persistence of *L. monocytogenes* (Mataragas et al. 2006, Zhang et al. 2012).

Antibiotic therapy is the most effective way to treatment the cases of food-borne listeriosis but high presence of antibiotic resistant strains of this bacterium cause hardness and prologues in treatment period and usually occur severe economic losses (Camargo et al. 2015, Dehkordi et al. 2013, Granier et al. 2011, Morvan et al. 2010). In keeping with this, an epidemiological research revealed that the *L. monocytogenes* strains of animal origins had the ranges of 10-80 percent resistance against commonly used antibiotics (Dehkordi et al. 2013). High prevalence of antibiotic resistance and increasing in the consumers' concerns regarding food security and consuming foods without any chemical preservative, led the food industries to use from the traditional plant extracts to reduce the microbial load of various types of foods (Kang et al. 2011). Now a days, application of medicinal plants and especially grape pulp, savory, and lemon due to their high antimicrobial effects and also complete contents of flavonols, antioxidants, phenolic acids, catkins, proantocianidine, anthocyanins, catkins, folic acids and other phenolic components have been increased in the food industries (Mahboubi and Kazempour 2011, Oliveira et al. 2013). However, using from medicinal planst in food industries requires more extensive research.

The aim of this study was to evaluate the antimicrobial effect of grape pulp, Bakhtiari savory, and lemon extracts on *L. monocytogenes* isolated from Iranian meat samples.

MATERIALS AND METHODS

SAMPLES COLLECTION

Grape sample was prepared from Shiraz native RISH BABA as well as the pulp extract dewatered from a kind of lemon, an extracted savory was also selected for the study. To dry the samples in a large volume, followed by the dewatering and the pulp separation, the grape cores were kept for a week at 25 to 30 ° C in the shade with proper ventilation.

ESSENCE EXTRACTION AND PREPARATION

The dried grape cores were kept in cool, dry place until the time of experiments. 100 gr core was weighed in order to take the essence and extracting (Mahboubi and Kazempour 2011). In order to prepare the extracts, 96% ethanol was used. For this purpose, the extraction was performed from 100 grams of vegetable using ethanol solvent, followed by concentrating the samples and alcohol separation using *Rubia tinctorum* in a vacuum (Siriken et al. 2013). To investigate the effective dose which has antibacterial properties, different concentrations were made from the extracts and selected doses were applied according to the sources.

To provide the desired concentrations, 0.01 gram of the extract was weighted and poured into the test tube, then 96% Ethanol was added to the final volume of 10 ml, followed by mixing thoroughly, the solution is called the Mother solution. Then the concentrations of 1000, 500, 250, 125, 62, 31 and 16 mg/ml were prepared.

STUDY THE ANTIMICROBIAL EFFECTS

To investigate the antimicrobial effects of desired extracts, Hinton agar plates were used. To inoculate the bacteria, 100 µl of bacteria suspension was poured on already prepared solid medium. Then, the liquid was spread over the medium surface using a sterile cotton swab or a sterile Pasteur L-shaped pipette. It was done identically for all the plates. Then, plate disks impregnated with the extracts were placed on the plates using sterile forceps, in the following, the antimicrobial properties were evaluated after 24 hours. To evaluate the antimicrobial properties of extracts and to determine the Minimum Bacterial Concentration (MBC) and Minimum Inhibitory Concentration (MIC) of each extract, the concentrations of MBC and MIC were used for inoculating on food sample (meat). For

Table 1. The investigation of grape pulp, lemon and savory extracts on *Listeria monocytogenes* in the minced meat.

Extract	Inoculated bacteria	Bacterial counts in days after inoculation		
		3rd	7th	10th
Grape pulp	103	102 × 2.2	102 × 9.5	102 × 8.8
	106	104 × 1.4	104 × 6.6	104 × 8.6
Savory	103	101 × 8.3	101 × 4.5	102 × 2.5
	106	103 × 3.6	103 × 8.5	103 × 3.4
Lemon	103	102 × 9.7	102 × 1.9	103 × 8.1
	106	104 × 2.9	105 × 3.2	105 × 1.4

this purpose, beef minced meat with about 15% fat was selected and minced with a meat mincer which the cutting blade, spiral parts and etc, were washed well and disinfected with 96% alcohol and dried. The desired meat was packed in 10 g plastic bags and was sterilized in the presence of UV radiation for 3 hours. In the following, the meats were inoculated with 10^3 and 10^6 concentrations of *L. monocytogenes* in the both groups, and then the prepared concentrations of the extracts were injected into the meats carrying the bacteria. After 3, 7 and 10 days, 1 g of the desired meat was put into test tubes containing sterile distilled water and the desired concentrations were given to the medium, then the bacteria grown were counted after 24 hours.

STATISTICAL ANALYSIS

To analyze the data, the one-way analysis of variance was used, as well as Duncan's and Tukey's mean comparisons with 5% probability level using SPSS statistical software (Ver. 20).

RESULTS

In the present study, the antibacterial effect of Bakhtiari savory, grape pulp and lemon pulp on *L. monocytogenes* was examined in vitro and in the minced meat. The results of 5- 10, 6- 10 and 7- 10 dilutions during 3, 7 and 10 days following the counting of bacteria suggested the best inhibitory effect in the Bakhtiar savory, grape pulp and lemon extracts, respectively.

Also, Bakhtiari savory, grape pulp and the lemon extracts were prepared and the effect of MBC and MIC were evaluated. The results showed that the Bakhtiari Savory have the greatest inhibitory effect in vitro compared to the Grape pulp and lemon, respectively.

DISCUSSION

In general, the amount and the distribution of *L. monocytogenes* in nature and environment is very high. These bacteria can be found everywhere, from human and animal feces to the soil, water and the variety of foods such as raw meat, fruits, milk and their products (19 of 30). Therefore, human beings, as a member of nature, can easily be infected with the bacteria, certainly by exposing one of the above mentioned items and having special conditions (8 and 30). The main transmission way for human infection is through the consumption of contaminated foods. The use of chemical preservatives to increase food durability and to prevent the growth of pathogens has resulted in the increase of chronic and incurable diseases. Therefore, the consumers mainly purchase and prepare foods without

Table 2. The effect of grape pulp, lemon and savory extracts on *Listeria monocytogenes* in vitro conditions.

Samples / Dilution	1000	500	250	125	62.5	31	15
Grape pulp	16- 18- 18 MBC	17- 17- 17 MBC	15- 15-16 MIC	14- 15- 14 Growth	12- 13- 13 Growth	12- 13- 13 Growth	9- 9- 10 Growth
Savory	Growth MIC	Growth Growth	Growth Growth	Growth Growth	Growth Growth	Growth Growth	Growth Growth
Lemon	15- 15-16 MIC	14- 14- 15 MIC	13- 13- 14 MIC	14- 13- 14 Growth	8- 9- 9 Growth	7- 8- 8 Growth	Growth Growth

additives and preservatives or with natural and safe preservatives. In the meantime, in recent years, several studies have been conducted on the antibacterial and the antioxidant effects of plant seed extracts.

Ghasemi et al. (2009) showed that the essential oil of Thymus aenensis and other species of thyme (ecotype Elam) have antimicrobial activity against *L. monocytogenes* present in chicken. In this study, the minimum inhibition concentration for 50% growth inhibition <MIC against *L. monocytogenes* for Thymus daenensis and Thymus sp. Were 70.0 and 7.1 mg / ml, respectively (Mahboubi and Kazempour 2011). According to the Rasouli and colleagues (2006), the extract and the essential oil of Thymus erioealyx and T. Porlock inhibits the growth of *L. monocytogenes*. The essential oils and the extracts of some aromatic plants (such as the mint family, Lamiaceae) with a high percentage of Carvacrol and thymol, have good activity against some bacteria (Chedea et al. 2013). Zdenka and colleagues (2004) showed the effect of antibacterial activity of the ethanol extract derived from the pulp and the seed of grapefruit against *Staphylococcus aureus*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Candida albicans* and other flora and the results showed that the extract of grapefruit seed has positive effects on the mortality of microorganisms (Cvetnic and Vladimir-Knezevic 2004). Joseph et al. (2009) showed that grape skin has the highest antimicrobial activity compared to the seeds (Brown et al. 2009). Veronica et al. (2011) showed in a study the effectiveness of phenolic extract against gram-positive bacterium linens and reducing the growth of *E. coli* (Chedea et al. 2013).

Jalali et al. (1385), studied the antimicrobial effects of hydroalcoholic extract derived from eucalyptus, rosemary, thyme, chamomile and sage on *L. monocytogenes* and showed that only the eucalyptus has antibacterial effects against *L. monocytogenes*. In a study by Mena and his colleagues in Portugal (2004) on commercial food production, the contamination of *L. monocytogenes* was reported 7.17% in raw meat, 7.16% in raw milk, 6.12% in raw fish, 18.5% in flour and 12.9% in fresh vegetables (Mena et al. 2004). In this study, the Bakhtiari savory showed the greatest antimicrobial effect against *L. monocytogenes* with a high percentage of carvacrol. After the Savory Bakhtiari, the grape pulp with anthocyanins and flavonoids chemical compounds, and the lemon with limonene chemical compounds had the highest antimicrobial effect against *L. monocytogenes*, respectively. Their inhibitory effects were studied and approved by the MBC and MIC tests in vitro and in the minced meat.

CONCLUSION

The study showed that the Bakhtiari Savory extract and then the grape pulp had the best inhibitory effect on *L. monocytogenes* both in vitro and in food. On the other hand, it was shown that the Bakhtiari Savory could be effective as a preservative to increase meat durability and to prevent the growth of pathogens such as *L. monocytogenes*.

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