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Study of antibacterial effect of total hydroalcoholic extract of *Thymus daenensis* and *Lavandula officinalis* on Listeria monocytogenes, an agent of food spoilage, using microdilution

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ABSTRACT

Background: Listeria monocytogenes is an important bacterial agent of food spoilage and cause of listeriosis. Recently, plants have been increasingly considered as alternatives to chemical drugs for preserving foods. Therefore, it is highly important to study their antibacterial effects. This study was conducted to investigate the antibacterial effect of Thymus daenensis and Lavandula officinalis on L. monocytogenes. Extraction was done by maceration using ethanol and concentration performed by rotary concentrator. Minimum inhibitory concentration (MIC) was examined by broth micro-dilution according to McFarland Equivalence (CFU/mL 10^5). To determine minimum bactericidal concentration (MBC), all the wells without opacity were cultured on Mueller-Hinton agar. Total hydroalcoholic extract of T. daenensis and L. officinalis had a positive effect on L. monocytogenes and, at certain concentrations, could inhibit the bacterial growth. The best inhibitory effect on bacterial growth was obtained for T. daenensis extract at 16 µg/mL. Furthermore, this extract had the highest bactericidal effect on L. monocytogenes. Regarding the findings of this study, the extracts of T. daenensis and L. officinalis could be used as antibacterial agents in food and pharmaceutical industries.

Key words: Antibacterial effect, Thymus daenensis, Lavandula officinalis, Listeria monocytogenes

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INTRODUCTION

Bacteria are significant microbial agents and causes of food poisoning and food spoilage, directly affecting community health [1]. Nowadays, the diseases caused by contaminated foods are a main health problem in the world, even developed countries. Since microbial infections threaten humans' health seriously and have been historically threatening their lives, they have been Constantly seeking out substances and drugs to treat disease and relieve its complications [2-12]. However, excessive use of antibacterial drugs has led to increased resistance of many bacteria to different antibiotics [13]. In this regard, use of herbal drugs, as nature-based substances with lower risk than antibiotics, to treat bacterial infections and other diseases is particularly important and is increasingly searched [14-18]. Medicinal plants and their secondary compounds are also highly important because of playing a protective role against various diseases, pathogenic agents or plant parasites such as fungi, bacteria, and viruses [19-23].The components that exert antibacterial, antifungal, and antiviral properties in certain plant species are referred to as phytoalexins [24]. Recently, antibacterial effects of different essences and extracts have been widely

investigated, indicating their ability to inhibit the growth of a wide variety of microorganisms with comparable and even greater effects than chemical and synthetic drugs. Antibacterial plant-based components have many therapeutic properties, and not only help to treat infectious or other diseases but also often decrease many side effects due to antimicrobial agents [25]. Over the past 25 years, listeriosis has been the most significant foodborne disease. The first epidemic of listeriosis occurred in 1981 due to cabbage salad. Immediately following epidemics were associated with cheese, cooked and prepared meat, chicken pieces, and fish. Currently, 2000 people are reported to acquire listerosis each year, of whom 500 die. L. monocytogenes is a weak competitor of other bacteria but grows rapidly in refrigerated conditions where other bacteria cannot compete. L. monocytogenes is highly dispersed in nature, is highly resistant to unfavorable environmental conditions, and is carried in feces of many people including healthy people, pregnant women, and patients with gastrointestinal disease. This infection may lead to complications such as meningitis and septicemia [26]. L. monocytogenes is found everywhere in nature such as soil, water, and plants and therefore is carried out by humans and animals in large quantities. This bacterium survives in dried stool for more than two years and in soil to 295 days. L. monocytogenes is resistant to drying and its survival depends on temperature. At lower temperatures, L. monocytogenes survives further in dry conditions. This which is important in food chain [27]. Nowadays, medicinal plants are considered as good substitutes for synthetic agents for treatment and prevention of various diseases, as well as for prevention of food spoilage Thymus daenensis Celak is one of these plants and is a perennial plant from Lamiaceae family and contains tannin, flavonoid, glycoside, caffeic acid, and rosmarinic acid [28]. T. daenensis has small, and oval leaves one cm in size, small and white or purple flowers as pillow bushes on wooden base. Usable part of this plant is flowering shoot. These shoots contain effective substances such as a purple essence with a completely distinguishable smell. T. daenensis has energizing, digesting, antispasmodic, antifungal, antibacterial, antiepileptic, and antioxidant properties [29]. Lavender, scientifically referred to as Lavandula officinalis, is a perennial plant, one miter in height. L. officinalis is leaves are narrow and crossed on stem. L. officinalis flowers are bluish purple and are integrated at the end of stem.[30,31] .Aerial organs of L. officinalis have been reported to contain essential components, flavonoid, tannin, and coumarine. L. officinalis essence has more than 40 different components. The most important components of this essence are linalool acetate, cineole, linalool, nerol, and borneol [32]. Given that medicinal plants occur widely in Iran, investigation of these plants for antimicrobial effects may help to provide appropriate nature-based alternatives to control and treat bacterial infections [33-36]. This leads to less frequent use of chemical drugs and therefore fewer side effects [37]. This study was conducted to determine the antibacterial effects of T. daenensis and L. officinalis on L. monocytogenes.

MATERIAL AND METHODS

This study was conducted in Medical Plants Research Center of Shahrekord University of Medical. After the plants were provided, extraction was conducted using ethanol 70% and concentration performed by rotary evaporator. A concentrated and relatively dry extract of T. daenensis and L. officinalis was obtained. In addition L. monocytogenes (Listeria monocygenes PTCC1163) bacteria was provided from Iran Scientific and Industrial Research and cultured according to the manufacturer's instructions. Bacterial suspension was prepared according to McFarland Equivalent (CFU/mL 10^5) Broth microdilution Minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) of T. daenensis and L. officinalis were determined by Broth microdilution. To prepare different dilutions, initially 80 mg of each extract (weighed by digital weight) was dissolved with one mL dimethyl sulfoxide 5% to achieve 80 mg/mL dilution. Then 10, 16, 20, 32, and 64 mg/mL dilutions of the extracts were prepared using Mueller-Hinton Broth medium. Then, 10 µL of the above bacterial suspensions were introduced to the wells. Only the first well (positive control) contained microbial suspension and Mueller-Hinton Broth medium and the second one (negative control) contained Mueller-Hinton Broth and the extracts.Then, the samples were incubated at 37°C for 24 hours. To determine MIC, the concentration of last (most diluted) well with no opacity was considered MIC, and to determine MBC, all the wells without opacity were cultured on blood agar medium and then incubated at 37°C for 24 hours. The lowest concentration in which the bacterial growth was inhibited was considered MBC.

RESULTS AND DISCUSSION

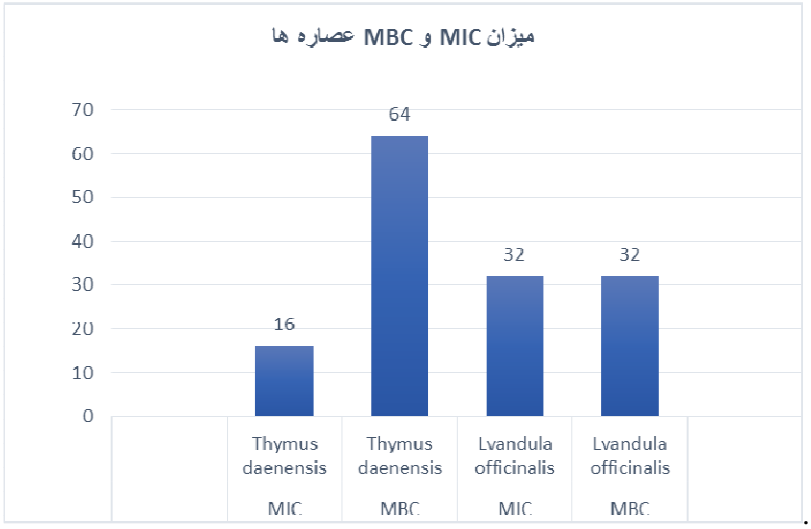
In this study, the lowest MIC (16 µg/mL) was obtained for T. daenensis extract. The highest MIC against L. monocytogenes (32 µg/mL) was obtained for L. officinalis extract. The lowest and highest MBC (32 and 64 µg/mL, respectively) was obtained for L. officinalis and T. daenensis extracts, respectively (Table 1). Total hydroalcoholic

extracts of T. daenensis and L. officinalis had great effects on L. monocytogenes. T. daenensis extract exerted optimal effect on L. monocytogenes.

Table 1. MIC * (µg/mL) and MBC ** (µg/mL) of the extracts

MBC (µg/mL)	MIC(µg/mL)	Extract
64	16	Thymus daenensis
32	32	Lavandula officinalis

**Minimum inhibitory concentration, ** minimum bactericidal concentration*



Figuren 1.Amount MIC and MBC extracts

This study was conducted to determine antibacterial effect of T. daenensis and L. officinalis on L. monocytogenes. The findings indicated that total hydroalcoholic extracts of T. daenensis and L. officinalis had positive effects on L. monocytogenes and, at certain concentrations, were able to inhibit bacterial growth. Furthermore, 16 µg/mL of L. officinalis had the best MIC. T. digenesis extract exerted the greatest bactericidal effect on L. monocytogenes, as well. Yin et al study on antibacterial effects of Allium hertifolium found that this plant inhibited the growth of four important foodborne bacterial species, L. monocytogenes, Staphylococcus aureus, Salmonella, and Escherichia coli O: 157 H:7. Several factors could contribute to antibacterial effects of this plant. An important explanation of inhibitory effects of plant extracts on bacteria may be phenolic components of these extracts, enabling them to affect decomposition of lipids of cell membrane and mitochondria and changes in membrane permeability and therefore bacterial cell death.Therefore, extract of a medicinal plant is likely to affect a microorganism greatly while it exerts lower or no effect on another microorganism and other diseases agents [38-60]. Method of extraction and type of used solvent may affect antibacterial effects of plants. If prepared by different methods and using different solvents, the extract of a plant may exert different antibacterial effects [57]. Sarabert investigated antibacterial effects of some plant extracts, at 0.2-10 µM/mL concentrations, on L. monocytogenes, Salmonella typhimurium, S. aureus, and Shigella dysenteriae and determined MIC of the extracts against these bacteria.

CONCLUSION

This study indicated that T. daenensis and L. officinalis total hydroalcoholic extract exerted considerable antibacterial effects. Because the antibiotic resistance of bacteria is constantly increasing, this finding is particularly important and these plants extracts could be used in pharmaceutical, food, and healthcare industries.

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