

Evaluation of Antibacterial Activity of *Satureja Khuzestanica* J. Essential Oil against Standard and Isolated Strains of *Listeria monocytogenes*

Sheida Akbari-Shahabi,*¹ Mehdi Assmar,¹ Alireza Massiha,¹ Naser Ghaemi,² Khosro Issazadeh,¹ Soheil Shokri-Fashtali³

1. Department of Microbiology, Lahijan Branch, Islamic Azad University, Lahijan, Iran
2. Department of Biotechnology, Tehran University, Tehran, Iran
3. Department of Mathematics, Lahijan Branch, Islamic Azad University, Lahijan, Iran

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*Corresponding author at:

Department of Microbiology,

Lahijan Branch, Islamic Azad

University, Lahijan, Iran.

E-mail:

Sheidaakbari.shahabi@yahoo.com

Abstract

Background: The purpose of this study was to determine antibacterial activity of essential oil of *Satureja khuzestanica* against *Listeria monocytogenes* (PTCC1295) and strains isolated from breast milk show that.

Materials and Methods: In this descriptive-analytic study, Essence of leave's plant was extracted and identified its compounds and then carvacrol was isolated. Antibacterial activities were examined by agar dilution method against *L. monocytogenes*. Minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) were carried out by micro dilution method. Then bacterial suspension injected the BALB/c mice. Forty-eight h after seeing the listeriosis disease signs were started the treatment. Ampicillin (10 µg/disc) and trimethoprim (5 g) were used as controls.

Results: The results showed that the inhibitory zone diameter standard and essential oils for strains isolated species were respectively 59 and 50 mm. This amount was determined by carvacrol, respectively, 60 and 48 mm. Inhibition zone diameter measurements for standard strains of ampicillin and trimethoprim tedious strains, respectively, 21, 40, 18 and 33 mm, respectively. The minimum inhibitory concentration of essential oils, carvacrol and ampicillin than standard strains, respectively 1.56, 1.56 and 155×10^{-8} µg/mL and MBC 3.125, 3.125 and 125×10^{-7} µg/mL was determined by the ratio of the strain 3.125, 3.125 and 0.0062 µg/mL and MBC was 6.25, 6.25 and 0.025 µg/mL.

Conclusion: This study showed that bacterial cleansing properties of essential oil of this plant have a strong and effective combination that is carvacrol.

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Introduction

Listeria monocytogenes causes listeriosis, which is the only species of human pathogens in immunocompromised individuals, infants and the fetus [1]. Food poisoning caused by bacteria, leading to complications such as meningitis, septicemia and abortion of pregnant women. In the epidemic, the mortality rate to 20% and 75% in people predisposed to it. When listeria meningitis occurs, the overall mortality may be as high as 70%; from septicemia 50%, from perinatal/neonatal infections greater than 80% [2, 3]. An important feature of this species is its intracellular growth. Immunoglobulin's and complement components in defense against this pathogen may not have played a prominent role. This is due to microbial cell wall surface proteins within the epithelial cells and hepatocytes probability of acceptance increases [4].

Mortality due to listeriosis, a very low dose of bacteria to cause infection, abundant in nature, lots of food contamination and bacterial growth at low temperatures has led to many attempts to control bacteria in food, especially in industrialized countries should be done. Recent efforts have been directed to the health and economic harms to reduce foodborne illness [5, 6].

Today, due to the appearance of adverse side-synthetic compounds and their incompatibility with the researchers to use natural herbs and ingredients those are available [7].

Satureja khuzestanica J. belongs to the dark one native plants of Lamiaceae and is widely grown in the southern part of its distribution, and the more mountainous areas and on limestone is fractured [8]. Evidences of anti-viral, anti-bacterial and anti-fungal components of some essential oils have been reported. Many studies have been done to determine which of the groups or spatial structures of compounds characteristic of the manufacturer responsible for their antibiotic is essential. Spatial structure of the cis double bond in the trans-spatial structure of the antimicrobial activity is more severe and the most active functional groups in aliphatic alcohols (eg linalool) and phenols (thymol and eugenol) is a hydroxyl group [9].

The main compound carvacrol is essential. Carvacrol is a wide spectrum of antimicrobial activity against bacteria. When bacteria are exposed to carvacrol fact, ATP and intracellular potassium leakage from the cell membrane and the bacterial cell dies [10].

Materials and Methods

The study was a descriptive-analytic study of the aerial parts of *S. khuzestanica*. Savory collection was ground after being dried in the shade. The powder obtained in a one liter flask was then poured into 300 mL of distilled water was added using a Clevenger apparatus was extracted oil. The ratio of oil to a Tyrode buffer salts containing HEPES, NaCl, KCl, NaH_2PO_4 , H_2O and glucose mixture by filtration, and 0.4 μm sterile, both aqueous extract and essential oil were stored at 4°C throughout the experiments. Essential oil is complex chemical compounds. GC/MS analysis was shown that carvacrol is the basic compounds of *S. khuzestanica* (Fig. 1). To determine this concentration, it independent of the amount of 1 mL and poured into a container of pre-measured after 24 h incubation at 50°C and the dry weight was measured again. Mean of triplicate experiments was considered essential as dry weight per mL, and the concentration was equivalent to 0.9, respectively. The standard strain of *Listeria monocytogenes* (PTCC 1295) made from the Iranian Research Organization for Science and Technology opened in aseptic conditions and liquid medium (Muller Hinton Broth) MHB transfer at 37°C was incubated for 24 hours. To ensure the purity of the bacteria on the linear MHB medium of choice Chrome Differential agar containing special Saplymnt were cultured for 24 h were incubated in 37°C. For prepare suspension of bacteria a loopful of the bacterial culture from the slant was inoculated in the MH broth and incubated overnight at 37°C. Then was compared with 0.5 McFarland and diluted two fold with sterile MH broth to prepare 10^8 colony forming unit (UFC/mL). This study tests the dilution medium Muller Hinton Broth and Muller Hinton Agar medium for disk diffusion method was used. All media according to the manufacturer's instructions Merck (MERK-Germany) and developed using the device were autoclaved. To evaluate the antimicrobial susceptibility of *L. monocytogenes* essence and carvacrol, 500 μL of a suspension prepared from strains and strains isolated from milk, with a density of 1.5×10^6 bacteria mL of Muller Hinton Agar on the environment in three directions (vertical, horizontal and diagonal) were cultured, Blank discs containing 20 μL and 20 μL of essence and carvacrol were scattered in separate plates. Of DMSO as a negative control and the antibiotic ampicillin, trimethoprim and sulfamethoxazole was used to compare the antimicrobial effects. MIC essential oil and carvacrol were tested individually on the desired bacteria. For this purpose, we used the method of dilution broth micro. A

96-well plate was used in the shaking incubator. Therefore, in each well of the plate, 200 mL MHB medium with 10 μL of bacterial suspension at a density 106×5.1 cells mL and 20 mL of different concentrations (200, 100, 50, 25, 12.5, 6.25, 3.125, 1.562, 0.8, 0.781, 0.390, 0.195, 0.098) essence and carvacrol and various concentrations of ampicillin (0.05, 0.025, 0.125, 0.0062, 0.0031, ..., to 24 dilution) was prepared, was added. This test was repeated three times. After the incubation period the wells opacity colony counts and bacterial growth inhibition was evaluated. Well as the lowest concentration preventing bacterial growth was considered the MIC essential oil and carvacrol. MIC50 (50% reduction in growth in the number of bacteria in the control group) as well as the slide navbar determined. To evaluate and determine the MBC content of all wells on the medium MHA was cultured. The lowest dilution of the extract and carvacrol was no growth was considered as MBC. All experiments were repeated three times. The comparison of average zone diameters and the evaluation of extract antimicrobial effects were analyzed by SPSS-17 and *t*-test. In this experiment, $p < 0.05$ was statistically significant.

Results

The results showed that the inhibition zone diameter ampicillin (10 g/disc) and trimethoprim (5 g/disc) against standard strains 21 and 40 mm, respectively (Fig. 2) and clinical isolates of *L. monocytogenes* 18 and 33 mm respectively (Fig. 3). While this amount for standard strains and species were isolated against oil and carvacrol, respectively, 62, 60, 50 and 48 mm, respectively (Fig. 2 and 3). The results of the MIC essential oil and carvacrol against standard strains and clinical isolates, respectively, 1.56, 3.125, 1.56 and 3.125 $\mu\text{g/mL}$. MIC of ampicillin against standard strains and clinical isolates was respectively, 155×10^{-8} and 0.0062 $\mu\text{g/mL}$. In this study, MIC50 standard strains and clinical isolates essential for the 0.8 or 2 $\mu\text{g/mL}$, MIC50 carvacrol against standard strains and isolates of 0.8 and 2 micrograms mL ampicillin MIC50 for clinical isolates and the standard 775×10^{-9} and 0.0031 $\mu\text{g/mL}$, respectively. The MBC amount of oil than the standard strain and clinical isolates, respectively 1.3 and 25.6 $\mu\text{g/mL}$ and MBC carvacrol were 125.3 and 25.6 $\mu\text{g/mL}$ was determined. While antibiotic ampicillin against standard strains and clinically isolated, 125×10^{-7} and 0.025 $\mu\text{g/mL}$, respectively. The results of this study suggest that the bactericidal effect of the essential oil and carvacrol in *S. khuzestanica* herb mixture while ampicillin static bacteria show an effect (Table 1).

Table1. Comparison of MIC & MBC of essential oil and carvacrol to ampicillin on *Listeria monocytogenes*

Inhibitor and Bactericide	MIC					
	MIC ₅₀ Bacteri		MIC ₉₀ Bacteri		Bacteri	
	Standard	Isolated strain	Standard	Isolated strain	Standard	Isolated strain
Essential oil	0.8	2	1.56	3.125	3.125	6.25
Carvacrol	0.8	2	1.56	3.125	3.125	6.25
Ampicillin	775×10^{-9}	0.0031	155×10^{-8}	0.0066	175×10^{-7}	0.025

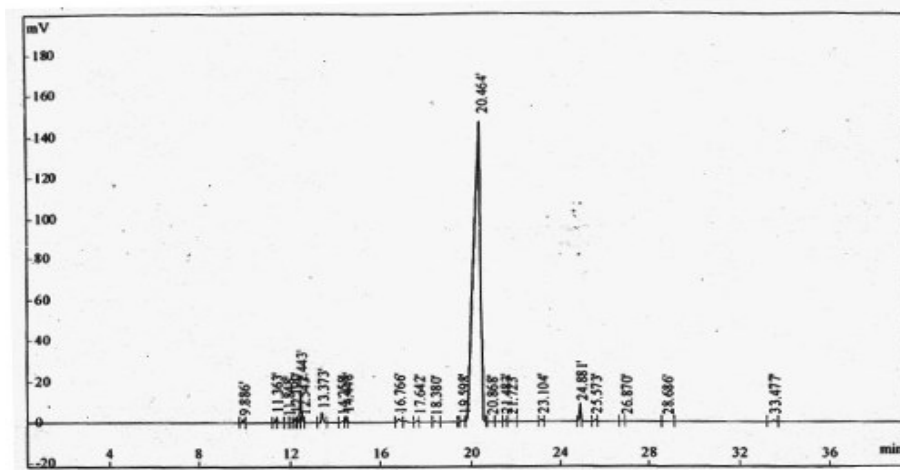


Figure 1. GC/MS analysis of the carvacrol compounds of *S. khuzestanica*

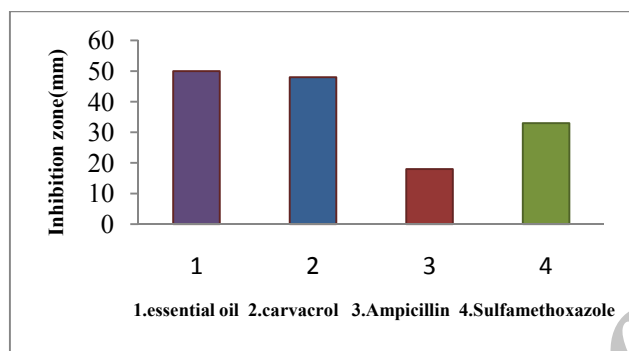


Figure 2. Antibacterial activity of essential oil and carvacrol of *S. khuzestanica*, ampicillin, sulfamethoxazole against standard strain of *L. monocytogenes*

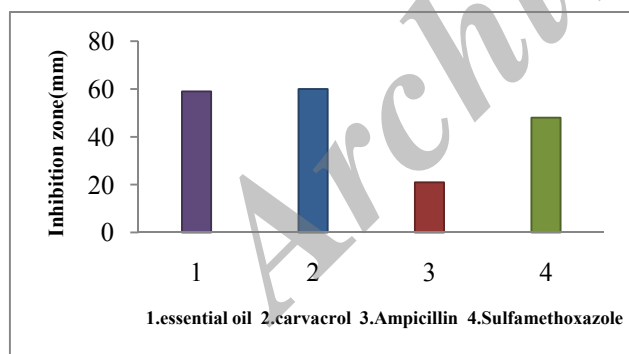


Figure 3. Antibacterial activity of essential oil and carvacrol of *S. khuzestanica*, ampicillin, sulfamethoxazole against isolated strain of *L. monocytogenes*

Discussion

In this study, phytochemical composition of essential oil obtained from the *S. khuzestanica* herb found that carvacrol has the highest chemical compounds found in plants. The antibacterial activity of essential oils and plant the impact carvacrol against standard strain and species isolated from milk was found that these two are the most lethal effect. While a mere sham controls showed an inhibitory effect. The antimicrobial properties of

medicines from medicinal plants have been distinguished since ancient times. Plants characterize an excellent source of new antimicrobial molecules. This plant is widely grown in south of Iran. *S. Khuzestanica* has therapeutic value because of uses as an analgesic and antiseptic in folk medicine [4, 11]. Many reports on antimicrobial activity and other properties of the plant there. In a study conducted by Majd et al., antibacterial activity of this plant extract on gram-positive bacteria, including *S. aureus* and *S. epidermidis* and Gram-negative bacteria include: *S. typhimorium*, *E. coli* and *P. auroginosa* and antifungal activity on the fungus *C. albicans* was shown in [12]. Zarrin et al., antifungal properties of this herb showed the *Cryptococcus neoformans* [9]. Sefidkon et al. study also confirmed bacterial plant [3]. Jalali et al. conducted a study that showed among the extracts of *Thymus vulgaris* L., *Eucalyptus globules*, *Matricaria recutita* L., *Rosmarinus officinalis* L., *Salvia officinalis* L., just *Eucalyptus globalus* with MIC=31.25 µg/mL has antibacterial activity against *Listeria monocytogenes* [13]. Baydar et al. showed antibacterial activity of essential oils depends on the type, composition and concentration of the oil, the type and concentration of the target microorganism, the composition of the substrate and the processing/storage conditions [11]. Compound and concentration of essential oil depend on environmental condition, plant creation stage, and growth and development stages. According to Majd et al. found that, most essential oils derived from plants before flowering and after flowering is the lowest. Carvacrol highest level of flowering is also related to the oil obtained from the plant after flowering has the least amount of carvacrol. Component of SKEO (before flowering stage) include carvacrol, γ -terpinene, paracymene, β -bisabolene, myrcene, α -thujene, α -pinene, terpinolene, thymyl acetate; but in a large quantity (92.87%) is carvacrol. In between carvacrol as original compositions in all developmental stages is essential. Maximum level before flowering and after flowering is at its lowest. During the flowerin stage increase p-cymene [12]. Carvacrol is an isothymol that inhibited activity of

ATPase enzymes; and to cause increasing nonspecific penetrable bacterial cell membrane, so increase microorganism sensiveness to entry extraneous matters [14, 15]. Given the evidence that anti-viral, anti-bacterial and anti-fungal components of some essential oils have been reported, many studies have been done to determine which of the groups or spatial structures essential ingredient manufacturer is responsible for its antibiotic properties requirements. Cis space structure of essential components, around the twofold linkage, to cause hyper antibacterial activity and hyper reaction group in aliphatic alcohols (like linalool) and phenols (like thymol) is hydroxyl group. Consideration of Majd et al. [12] different result of SKEO antibacterial properties on gram positive and gram negative bacterial, is because different structure of cell wall. All of the gram negative bacterial have lipopolyschacaride outer layer with hydrophil structure; so small and hydrophile solvate can pass the cell wall. But component of essential oil is hydrophobe, it is reason that gram negative bacterial are resistant against SKEO, so essential oil of *S. khuzestanica* to be effective against *L. monocytogenes* [5, 16]. According to the MIC reading the results of the disk-based rates in this study,

the antibacterial plant and acceptance of the current plant community several properties of these plants can be introduced and used in synthetic drugs to promoting public health in healthy individuals and in individuals with impaired immune systems helped.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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