# Investigating the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the *Lavandula stoechas* L. and *Rosmarinus officinalis* L. extracts on pathogen bacterias *"in vitro"*

**Farideh Tabatabaei Yazdi*, Behrooz Alizadeh Behbahani, Ali Mortazavi**

Department of Food Science and Technology, Ferdowsi University of Mashhad, Mashhad, Iran

*Corresponding Author: email address: tabatabai@um.ac.ir (F.Tabatabaei Yazdi)

## ABSTRACT

The infections risk related to pathogenic germs increases at the present time considering the increased resistance which certain microbes acquire, whose usual antibiotics are ineffective to treat the infectious disease. The aim of this study was to determine antimicrobial effect of the aqueous and ethanolic extracts of *Lavandula stoechas* L. and *Rosmarinus officinalis* L. on *Listeria monocytogenes* PTCC 1297 ،*Bacillus cereus* PTCC 1154، *Enterobacter aerogenes* PTCC 1221، *Enterococcus faecalis* PTCC 1237 and *Salmonella typhi* PTCC 1609 *"in vitro"*. In this experimental study, after collecting plants from of Razavi Khorasan province, the extraction was carried out by the maceration method, after antimicrobial effect of the extracts evaluated by two methods, "Collins method" (spreading of the extract on medium surface) and "disk agar diffusion method". The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) for both species determined by using a dilution method. Statistical analysis was carried out by analysis of variance (ANOVA).The results show that aqueous and ethanolic *Lavandula stoechas* L. extracts were quite effective in 2000 µg/ml concentration on *Listeria monocytogenes* ،*Bacillus cereus* and *Enterococcus faecalis*. The results indicate that ethanolic extracts of *Lavandula stoechas* L. have the greatest effect on gram-positive bacterium. The result shows that MIC of *Lavandula stoechas* L. leaves of the aqueous and ethanolic extracts for *Enterobacter aerogenes* was 32 and 16 mg/ml respectively. The result shows that MIC of *Rosmarinus officinalis* L. leaves of the aqueous and ethanolic extracts for *Enterobacter aerogenes* was 128 and 64 mg/ml respectively.The *Lavandula stoechas* L. and *Rosmarinus officinalis* L. extracts presented the more effective impact on the growth of gram-positive bacteria than gram-negative bacteria ($p<0.05$).

**Keywords**: Minimum Inhibitory Concentration, Minimum Bactericidal Concentration, Antimicrobial effects, Extract, *Lavandula stoechas* L., *Rosmarinus officinalis* L.

## INTRODUCTION

Rosemary (*Rosmarinus officinalis* L.) is a spice and medicinal herb widely used around the world. Of the natural antioxidants, rosemary has been widely accepted as one of the spices with the highest antioxidant activity [1]. Rosemary is known to have antioxidant and antibacterial properties. Putnam *et al*, (2006) reported that rosemary essential oil inhibit osteoclast activity and increase bone density "*in vitro"* [2]. Also, cytotoxic activity of rosemary essential oil has been demonstrated by several authors [3].

The Lavenders are a genus of about 25-30 species of flowering plants in the mint family, Lamiaceae, native to the Mediterranean region south to tropical Africa and to the many regions of Asia. The genus includes annuals, herbaceous plants, sub shrubs, and small shrubs [4]. Lavender has been used for centuries as an herbal remedy. Lavender yields a highly effective essential oil with very sweet overtones, and can be used in balms, salves, perfumes, cosmetics, and topical applications. Internally, Lavender essential oil is believed to be of benefit for a multitude of problems, including stress, anxiety, exhaustion, irritability, headaches, migraines, insomnia, depression, colds, digestion, flatulence, upset stomach, liver and gallbladder problems, nervousness, loss of

appetite, and as a breath freshener and mouthwash [5, 6].

Food market trends are changing. Consumers demand more high-quality foods with fresh like attributes; consequently less extreme treatments and/or additives are being required. Lipid oxidation and bacterial contamination are the main factors that determine the loss of food quality and shelf-life reduction. Therefore, delaying lipid oxidation and preventing bacterial cross contamination are highly relevant to food processors. Oxidative processes and bacterial contamination, in turn, contribute to the deterioration in flavor, texture and color of food products [7]. This is why that many diseases which we controlled formerly, have reappeared and escaped from human control. The antibacterial activities of essential oils from various medicinal plants against micro-organisms were described and proven in experiments by various researchers [8, 9].

The aim of this study was to determine antimicrobial effect of the aqueous and ethanolic extracts of *Lavandula stoechas L*. and *Rosmarinus officinalis L*. on *Listeria monocytogenes* PTCC 1297 ،*Bacillus cereus* PTCC 1154، *Enterobacter aerogenes* PTCC 1221، *Enterococcus faecalis* PTCC 1237 and *Salmonella typhi* PTCC 1609 *"in vitro"*.

## MATERIALS AND METHODS

***Preparation Plant***

In this study, the *Lavandula stoechas L.* and *Rosmarinus officinalis L.* purchased from local markets in Mashhad, Iran and the species were identifying in the herbarium of Ferdowsi University of Mashhad.

***Preparation of aqueous and ethanolic extracts from Lavandula stoechas L*. and *Rosmarinus officinalis L*.**

Maceration method was used to prepare extracts. The amount 100 gram of *Lavandula stoechas* L. and *Rosmarinus officinalis* L. leaves powder was Added to 500 ml ethanol 96 degree or distilled water. The ethanolic extract mixture was preserved at laboratory temperature for 24 hours and was stirred every few hours with a glass rod. The aqueous mixture was boiled for 20 minutes with low flame until the cream colored liquid was obtained. The collecting supernatant was centrifuged by 3000 rpm for 10 min. The resulting extract (supernatant) volume has reached to the original with ethanol or distilled water, and then samples were stored into the dark container at refrigerator temperature after filtering by 0.45 µ Whatman filter paper [10, 11].

***Determination dry weight of aqueous and ethanolic extracts Lavandula stoechas L*. and *Rosmarinus officinalis L*.**

At first the weight of a tube were measured, and then 1ml of aqueous and ethanolic extracts were poured in it. The contents of the tube were dried at room temperature. After drying the extract, the tubes were weighed again. Weight differences are equivalent weight of 1ml aqueous and ethanolic extracts. Average of three replicates, was calculated as the dry weight of the extract [12].

***Source of microorganisms***

The bacterial strain used *Listeria monocytogenes* PTCC 1297 ،*Bacillus cereus* PTCC 1154، *Enterobacter aerogenes* PTCC 1221، *Enterococcus faecalis* PTCC 1237 and *Salmonella typhi* PTCC 1609 for each test, to evaluating the antimicrobial effects, fresh medium was prepared.

***Preparation of Microbial suspension***

To preparing microbial suspensions, requires 24-hour culture from each microorganism. So, 24 hours before experiments, microorganisms were inoculated from storage medium to nutrient agar medium slope. After 24 hours, the cultures were washed by Ringer solution and microbial suspensions were prepared. Then some of the bacterial suspension was poured in sterile tubes containing Ringer solution and its turbidity, was measured by spectrophotometer at 530 nm wavelength. It was diluted by Ringer solution until the solution turbidity equalizes with 5% McFarland standard solution. Suspension should have contains $1.5\times 10^8$ CFU / ml (13).

***Evaluation of antimicrobial activity***

Adding extracts to the culture medium "according of the method of Collins *et al.* (1995)" and "disk agar diffusion method" were done and to evaluated the antimicrobial effects of alcoholic *Lavandula stoechas L*. and *Rosmarinus officinalis L*. extracts [14].

Then 0.2 gram of ethanol extract, were added to 5 ml of sterile distilled water. Then it was stirred with vortex system to assist being steady. Then 1 ml of this solution was added to sterile plates. The final concentration of the extract was 2000 µg /ml [15]. In the next step, Mueller Hinton Agar (Merck-Germany) medium were sterile and used for bacteria, were added to the plates, and placed at room temperature, so the medium was prepared. One loop of each standard strain culture media was cultured inoculums on these medium. The plates were incubated for 48 hours at 37 ° C. The culture with extract and without bacteria was used as control [15]. The disk agar diffusion method, at first a loop of each standard strain culture media was cultured on the plates, and then paper discs (from Whatman filter with 6 mm diameter), plates were saturated with 100 µl of the test compound allowed to dry and was introduced on the upper layer of the seeded agar plate with 5, 10, 15, 20, 25, 30, 35 and 40 mg/ml extract concentrations, were prepared in distilled water and was treated with *Lavandula stoechas L*. and *Rosmarinus officinalis L*. extract and placed in culture medium by Sterile loop. Then it was fixed on the media with a light little pressure. After incubation time, the diameter of free zone was measured exactly by using a ruler in millimeters. All experiments were performed with 3 replicates [16].

***Determination of Minimum Inhibitory Concentration (MIC)***

MIC was determined according to agar dilution method [17]. Various concentrations of extract were prepared in 10 cm experimental tubes containing Mueller Hinton Broth for fungi and 10 cm experimental tubes containing Mueller Hinton Broth for bacteria. Each tube contains 9 ml of Mueller Hinton for bacteria were sterilized by autoclaving. On cooling, 1 ml of each extract (watery & etanoli) concentration were added to each tube, to make the final concentrations of 2, 4, 8, 16, 32, 64, 128, 256 mg/ml. The mixture of Muller Hilton and extract was poured into plates aseptically in a laminar flow cabinet. On solidification of the agar medium, 2 µl of adjusted spore suspension were added to each plate by micropipette and incubated at 37 C° for bacteria [18]. The Muller Hilton without any herbal extract served as control. The MIC was regarded as the lowest concentration of the extract that did not show any visible growth after 24 hours of incubation (compared with control).

***Determination of Minimum Bactericidal Concentration (MBC)***

The in vitro MBC were determined for each extracts (watery & etanoli) as previously described with slight modifications [19]. The MBC was determined by incorporating various concentrations of extracts (of 2, 4, 8, 16, 32, 64, 128, 256 mg/ml) in Muller Hilton Broth in tubes for bacteria. One milliliter adjusted spore suspension was added to each tube and incubated at 37°C for 2 days.

***Statistical analysis***

All the assays were carried out in triplicates. The experimental results were expressed as mean ± standard deviation. The data were analyzed using one way analysis of variance (ANOVA) using SPSS version 17. One-way analysis of variance (ANOVA) was used to analyze each treatment over storage. In the case of significant differences, Tukey test ($p<0.05$) was used.

## RESULTS

The results of the antimicrobial effects of aqueous and ethanolic extracts, by using the method of Collins *et al.* were show on in Tables 1 and 2. The results showed 2000 µg/ml concentration of both aqueous and ethanolic *Lavandula stoechas* L. extracts, were quite effective on reduce of growth *Listeria monocytogenes* ،*Bacillus cereus* ، *Enterococcus faecalis* and *Salmonella typhi* were had prevent growth over the medium. However, 2000 µg/ ml concentration aqueous and ethanolic extracts, have no significant antibacterial effect on *Enterobacter aerogenes* and it is not able to prevent the growth of bacteria on culture. The results showed 2000 µg/ml concentration of both aqueous and ethanolic *Rosmarinus officinalis* L. extracts, were quite effective on reduce of growth *Listeria monocytogenes* ،*Bacillus cereus* and *Enterococcus faecalis* were had prevent growth over the medium. However, 2000 µg/ml concentration aqueous and ethanolic extracts, have no significant antibacterial effect on *Salmonella typhi and Enterobacter aerogenes* and it is not able to prevent the growth of bacteria on culture.

The results of the antimicrobial effects of aqueous and ethanolic *Lavandula stoechas* L. and *Rosmarinus officinalis* L. extracts, by the agar diffusion method are presented in Tables 3 and 4. The results show that *Lavandula stoechas* L. ethanolic extract at all concentrations (5, 10, 15, 20, 25, 30, 35 and 40 mg/ml) had the inhibitory effect on *Listeria monocytogenes* ،*Bacillus cereus*، *Salmonella typhi* and *Enterococcus faecalis*، However, 5 mg/ml concentration extracts, have no significant antimicrobial effect on *Enterobacter aerogenes* and it is not able to prevent the growth of bacteria on culture ($p<0.05$). The results show that *Rosmarinus officinalis* L. ethanolic extract at all concentrations (5, 10, 15, 20, 25, 30, 35 and 40 mg/ml) had the inhibitory effect on *Listeria monocytogenes* ،*Bacillus cereus* and *Enterococcus faecalis*، However, 5 and 10 mg/ml concentration extracts, have no significant antimicrobial effect on *Salmonella typhi and Enterobacter aerogenes* and it is not able to prevent the growth of bacteria on culture ($p<0.05$). The results show that *Lavandula stoechas* L. aqueous extract at all concentrations (5, 10, 15, 20, 25, 30, 35 and 40 mg/ml) had the inhibitory effect on *Listeria monocytogenes* ،*Bacillus cereus* and *Enterococcus faecalis* However, 5, 10 and 15 mg/ml concentration extracts, have no significant antimicrobial effect on *Salmonella typhi and Enterobacter aerogenes* and it is not able to prevent the growth of bacteria on culture ($p<0.05$). The results show that *Rosmarinus officinalis* L. aqueous extract at all concentrations (5, 10, 15, 20, 25, 30, 35 and 40 mg/ml) had the inhibitory effect on *Listeria monocytogenes* and *Enterococcus faecalis*، However, 5 and 10 mg/ml concentration extracts, have no significant antimicrobial effect on *Bacillus cereus and* 5, 10, 15 and 20 mg/ml concentration extracts, have no significant antimicrobial effect on *Salmonella typhi and Enterobacter aerogenes* and it is not able to prevent the growth of bacteria on culture ($p<0.05$).

MIC results of the aquous and ethanolic extract of *Lavandula stoechas* L. are given in Table 5. The results shows that MIC of ethanolic extract of *Lavandula stoechas* L. for used *Listeria monocytogenes*، *Bacillus cereus*، *Enterococcus faecalis*، *Salmonella typhi* and *Enterobacter aerogenes* was 2, 4, 4, 8 and 16 mg/ml respectively. The results shows that MIC of aquous extract of *Lavandula stoechas* L. for used *Listeria monocytogenes*، *Bacillus cereus*، *Enterococcus faecalis*، *Salmonella typhi* and *Enterobacter aerogenes* was 4, 4, 8, 16 and 32 mg/ml respectively.

MIC results of the aquous and ethanolic extract of *Rosmarinus officinalis* L. are given in Table 6. The results shows that MIC of ethanolic extract of *Lavandula stoechas* L. for used *Listeria monocytogenes*، *Bacillus cereus*، *Enterococcus faecalis*، *Salmonella typhi* and *Enterobacter aerogenes* was 8, 8, 16, 32 and 64 mg/ml respectively. The results shows that MIC of aquous extract of *Rosmarinus officinalis* L. for used *Listeria monocytogenes*، *Bacillus cereus*، *Enterococcus faecalis*، *Salmonella typhi* and *Enterobacter aerogenes* was 8, 16, 32, 64 and 128 mg/ml respectively.

MBC results of the aquous and ethanolic extract of *Lavandula stoechas* L. are given in Table 7. The results shows that MBC of ethanolic extract of *Lavandula stoechas* L. for used *Listeria monocytogenes*، *Bacillus cereus*، *Enterococcus faecalis*، *Salmonella typhi* and *Enterobacter aerogenes* was 4, 16, 16, 32 and 32 mg/ml respectively. The results shows that MBC of aquous extract of *Lavandula stoechas* L. for used *Listeria monocytogenes*، *Bacillus cereus*، *Enterococcus faecalis*، *Salmonella typhi* and *Enterobacter aerogenes* was 4, 32, 32, 64 and 128 mg/ml respectively.

MBC results of the aquous and ethanolic extract of *Rosmarinus officinalis* L. are given in Table 8. The results shows that MBC of ethanolic extract of *Lavandula stoechas* L. for used *Listeria monocytogenes*، *Bacillus cereus*، *Enterococcus faecalis*، *Salmonella typhi* and *Enterobacter aerogenes* was 16, 16, 64, 128 and 128 mg/ml respectively. The results shows that MBC of aquous extract of *Rosmarinus officinalis* L. for used *Listeria monocytogenes*، *Bacillus cereus*، *Enterococcus faecalis*، *Salmonella typhi* and *Enterobacter aerogenes* was 32, 32, 64, 128 and 256 mg/ml respectively.

**Table 1**: Antimicrobial effects of 2000µg/ml ethanolic and aqueous *Lavandula stoechas* L. extract, on *Listeria monocytogenes*, *Bacillus cereus*, *Enterococcus faecalis*, *Salmonella typhi* and *Enterobacter aerogenes*

| **Microorganism** | **Antimicrobial effects of aqueous *Lavandula stoechas* L. extract** |
|---|---|
| ***Listeria monocytogenes* PTCC 1297** | + |
| ***Bacillus cereus* PTCC 1154** | + |
| **Enterococcus faecalis PTCC 1237** | + |
| ***Salmonella typhi* PTCC 1609** | + |
| ***Enterobacter aerogenes* PTCC 1221** | _ |

| **Microorganism** | **Antimicrobial effects of ethanolic *Lavandula stoechas* L. extract** |
|---|---|
| ***Listeria monocytogenes* PTCC 1297** | + |
| ***Bacillus cereus* PTCC 1154** | + |
| **Enterococcus faecalis PTCC 1237** | + |
| ***Salmonella typhi* PTCC 1609** | + |
| ***Enterobacter aerogenes* PTCC 1221** | _ |

- (+) in Table showed no bacterial growth on culture and antibacterial activity of aqueous ane ethanolic *Lavandula stoechas* L.extract
- (-) in Table showed the growth of bacteria on culture and the lack of antibacterial activity of aqueous and *Lavandula stoechas* L.extract

**Table 2**: Antimicrobial effects of 2000µg/ml ethanolic and aqueous *Rosmarinus officinalis* L. extract, on *Listeria monocytogenes*, *Bacillus cereus*, *Enterococcus faecalis*, *Salmonella typhi* and *Enterobacter aerogenes*

| **Microorganism** | **Antimicrobial effects of aqueous *Rosmarinus officinalis* L. extract** |
|---|---|
| ***Listeria monocytogenes* PTCC 1297** | + |
| ***Bacillus cereus* PTCC 1154** | + |
| ***Enterococcus faecalis* PTCC 1237** | + |
| ***Salmonella typhi* PTCC 1609** | _ |
| ***Enterobacter aerogenes* PTCC 1221** | _ |

| **Microorganism** | **Antimicrobial effects of ethanolic *Rosmarinus officinalis* L. extract** |
|---|---|
| ***Listeria monocytogenes* PTCC 1297** | + |
| ***Bacillus cereus* PTCC 1154** | + |
| **Enterococcus faecalis PTCC 1237** | + |
| ***Salmonella typhi* PTCC 1609** | _ |
| ***Enterobacter aerogenes* PTCC 1221** | _ |

- (+) in Table showed no bacterial growth on culture and antibacterial activity of aqueous ane ethanolic *Rosmarinus officinalis* L. extract
- (-) in Table showed the growth of bacteria on culture and the lack of antibacterial activity of aqueous and ethanolic *Rosmarinus officinalis* L. extract

**Table 3**: Average diameter (mm) of microbial free zone area of ethanolic and aqueous *Lavandula stoechas* L. extract concentrations on *Listeria monocytogenes*, *Bacillus cereus*, *Enterococcus faecalis*, *Salmonella typhi* and *Enterobacter aerogenes* (disk agar diffusion method).

| | | ***Lavandula stoechas* L. extract concentrations (mg/ml)** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Extract** | **Microorganism** | **5** | **10** | **15** | **20** | **25** | **30** | **35** | **40** |
| **ethanolic** | ***Listeria monocytogenes*** | 9.60±0.28[a] | 11.30±0.54[b] | 13.50±0.57[c] | 15.20±0.28[d] | 17.20±0.50[e] | 18.90±0.28[f] | 19.90±0.50[g] | 22.10±0.28[h] |
| **ethanolic** | ***Bacillus cereus*** | 8.20±0.54[a] | 9.90±0.57[b] | 11.40±0.28[c] | 13.00±0.50[d] | 15.10±0.57[e] | 17.00±0.50[f] | 18.40±0.57[g] | 19.70±0.54[h] |
| **ethanolic** | ***Enterococcus faecalis*** | 8.00±0.50[a] | 9.10±0.28[b] | 10.90±0.50[c] | 11.30±0.54[d] | 13.90±0.28[e] | 15.90±0.50[f] | 16.70±0.54[g] | 18.30±0.50[h] |
| **ethanolic** | ***Salmonella typhi*** | 7.10±0.28[a] | 8.30±0.54[b] | 9.90±0.50[c] | 11.80±0.54[d] | 13.10±0.54[e] | 15.00±0.54[f] | 16.80±0.28[g] | 18.00±0.20[h] |
| **ethanolic** | ***Enterobacter aerogenes*** | – | 7.30±0.28[a] | 8.90±0.28[b] | 10.10±0.50[c] | 11.60±0.50[d] | 13.40±0.50[e] | 14.90±0.54[f] | 15.50±0.50[g] |
| **aqueous** | ***Listeria monocytogenes*** | 8.50±0.50[a] | 9.90±0.50[b] | 12.00±0.54[c] | 13.90±0.57[d] | 15.20±0.20[e] | 17.00±0.20[f] | 18.90±0.28[g] | 20.00±0.50[h] |
| **aqueous** | ***Bacillus cereus*** | 7.60±0.28[a] | 8.60±0.54[b] | 10.00±0.57[c] | 11.90±0.28[d] | 13.40±0.28[e] | 14.90±0.50[f] | 17.10±0.54[g] | 18.60±0.54[h] |
| **aqueous** | ***Enterococcus faecalis*** | 6.90±0.28[a] | 8.00±0.50[b] | 9.50±0.50[c] | 11.00±0.20[d] | 12.20±0.54[e] | 13.10±0.50[f] | 15.00±0.52[g] | 16.80±0.50[h] |
| **aqueous** | ***Salmonella typhi*** | – | – | – | 7.00±0.50[a] | 8.80±0.57[b] | 9.90±0.57[c] | 11.20±0.28[d] | 12.90±0.50[e] |
| **aqueous** | ***Enterobacter aerogenes*** | – | – | – | 6.50±0.50[a] | 7.80±0.50[b] | 10.00±0.54[c] | 11.00±0.28[d] | 12.20±0.50[e] |

**Table 4**: Average diameter (mm) of microbial free zone area of ethanolic and aqueous *Rosmarinus officinalis* L. extract concentrations on *Listeria monocytogenes*, *Bacillus cereus*, *Enterococcus faecalis*, *Salmonella typhi* and *Enterobacter aerogenes* (disk agar diffusion method).

| | | ***Rosmarinus officinalis* L. extract concentrations (mg/ml)** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Extract** | **Microorganism** | **5** | **10** | **15** | **20** | **25** | **30** | **35** | **40** |
| **ethanolic** | ***Listeria monocytogenes*** | 8.50±0.54[a] | 9.90±0.50[b] | 11.00±0.50[c] | 12.90±0.28[d] | 14.80±0.54[e] | 17.00±0.20[f] | 18.70±0.54[g] | 20.60±0.50[h] |
| **ethanolic** | ***Bacillus cereus*** | 7.50±0.28[a] | 8.50±0.57[b] | 10.70±0.57[c] | 11.40±0.54[d] | 13.10±0.57[e] | 15.00±0.50[f] | 17.60±0.57[g] | 18.00±0.50[h] |
| **ethanolic** | ***Enterococcus faecalis*** | 7.30±0.50[a] | 8.80±0.54[b] | 10.00±0.57[c] | 11.80±0.50[d] | 13.00±0.50[e] | 14.90±0.54[f] | 16.00±0.50[g] | 17.30±0.28[h] |
| **ethanolic** | ***Salmonella typhi*** | _ | _ | 7.90±0.50[a] | 9.00±0.54[b] | 11.10±0.28[c] | 12.70±0.28[d] | 14.00±0.50[e] | 15.80±0.54[f] |
| **ethanolic** | ***Enterobacter aerogenes*** | – | _ | 7.00±0.54[a] | 8.60±0.50[b] | 10.10±0.50[c] | 12.00±0.50[d] | 13.60±0.54[e] | 15.20±0.28[f] |
| **aqueous** | ***Listeria monocytogenes*** | 8.00±0.28[a] | 9.40±0.54[b] | 10.10±0.28[c] | 12.60±0.57[d] | 13.20±0.28[e] | 15.90±0.28[f] | 17.80±0.50[g] | 18.30±0.54[h] |
| **aqueous** | ***Bacillus cereus*** | _ | _ | 8.00±0.50[a] | 10.10±0.54[b] | 11.90±0.20[c] | 13.00±0.20[d] | 15.40±0.28[e] | 16.90±0.56[f] |
| **aqueous** | ***Enterococcus faecalis*** | 6.50±0.50[a] | 7.40±0.57[b] | 8.90±0.28[c] | 10.10±0.54[d] | 11.80±0.57[e] | 12.20±0.54[f] | 13.90±0.52[g] | 16.00±0.20[h] |
| **aqueous** | ***Salmonella typhi*** | – | – | – | _ | 8.00±0.54[a] | 10.10±0.20[b] | 11.00±0.50[c] | 12.10±0.54[d] |
| **aqueous** | ***Enterobacter aerogenes*** | – | – | – | _ | 7.00±0.20[a] | 8.80±0.50[b] | 10.10±0.50[c] | 11.70±0.20[d] |

[a] Values are means ± standard deviations, n=3.

(-) in Table showed no inhibitory effects was shown

**Table 5**: Minimum Inhibitory Concentration (MIC) of ethanolic and aqueous *Lavandula stoechas* L. extract on *Listeria monocytogenes, Bacillus cereus, Enterococcus faecalis, Salmonella typhi* and *Enterobacter aerogenes*

| | | | | ***Lavandula stoechas* L. extract concentrations (mg/ml)** | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Extract** | **Microorganism** | **2** | **4** | **8** | **16** | **32** | **64** | **128** | **Control** |
| **ethanolic** | ***Listeria monocytogenes*** | + | + | + | + | + | + | + | − |
| **ethanolic** | ***Bacillus cereus*** | − | + | + | + | + | + | + | − |
| **ethanolic** | ***Enterococcus faecalis*** | − | + | + | + | + | + | + | − |
| **ethanolic** | ***Salmonella typhi*** | − | − | + | + | + | + | + | − |
| **ethanolic** | ***Enterobacter aerogenes*** | − | − | − | + | + | + | + | − |
| **aqueous** | ***Listeria monocytogenes*** | − | + | + | + | + | + | + | − |
| **aqueous** | ***Bacillus cereus*** | − | + | + | + | + | + | + | − |
| **aqueous** | ***Enterococcus faecalis*** | − | − | + | + | + | + | + | − |
| **aqueous** | ***Salmonella typhi*** | − | − | − | + | + | + | + | − |
| **aqueous** | ***Enterobacter aerogenes*** | − | − | − | − | + | + | + | − |

+: Positive inhibition
- : Negative inhibition

**Table 6**: Minimum Inhibitory Concentration (MIC) of ethanolic and aqueous *Rosmarinus officinalis* L. extract on *Listeria monocytogenes, Bacillus cereus, Enterococcus faecalis, Salmonella typhi* and *Enterobacter aerogenes*

| | | | | ***Rosmarinus officinalis* L. extract concentrations (mg/ml)** | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Extract** | **Microorganism** | **2** | **4** | **8** | **16** | **32** | **64** | **128** | **Control** |
| **ethanolic** | ***Listeria monocytogenes*** | − | − | + | + | + | + | + | − |
| **ethanolic** | ***Bacillus cereus*** | − | − | + | + | + | + | + | − |
| **ethanolic** | ***Enterococcus faecalis*** | − | − | − | + | + | + | + | − |
| **ethanolic** | ***Salmonella typhi*** | − | − | − | − | + | + | + | − |
| **ethanolic** | ***Enterobacter aerogenes*** | − | − | − | − | − | + | + | − |
| **aqueous** | ***Listeria monocytogenes*** | − | − | + | + | + | + | + | − |
| **aqueous** | ***Bacillus cereus*** | − | − | − | + | + | + | + | − |
| **aqueous** | ***Enterococcus faecalis*** | − | − | − | − | + | + | + | − |
| **aqueous** | ***Salmonella typhi*** | − | − | − | − | − | + | + | − |
| **aqueous** | ***Enterobacter aerogenes*** | − | − | − | − | − | − | + | − |

- +: Positive inhibition
- - : Negative inhibition

**Table 7**: Minimum Bacterial Concentration (MBC) of ethanolic and aqueous *Lavandula stoechas* L. extract on *Listeria monocytogenes*, *Bacillus cereus*, *Enterococcus faecalis*, *Salmonella typhi* and *Enterobacter aerogenes*

| | | | | ***Lavandula stoechas* L. extract concentrations (mg/ml)** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Extract** | **Microorganism** | **2** | **4** | **8** | **16** | **32** | **64** | **128** | **256** | **control** |
| **ethanolic** | ***Listeria monocytogenes*** | − | + | + | + | + | + | + | + | − |
| **ethanolic** | ***Bacillus cereus*** | − | − | − | + | + | + | + | + | − |
| **ethanolic** | ***Enterococcus faecalis*** | − | − | − | + | + | + | + | + | − |
| **ethanolic** | ***Salmonella typhi*** | − | − | − | − | + | + | + | + | − |
| **ethanolic** | ***Enterobacter aerogenes*** | − | − | − | − | + | + | + | + | − |
| **aqueous** | ***Listeria monocytogenes*** | − | + | + | + | + | + | + | + | − |
| **aqueous** | ***Bacillus cereus*** | − | − | − | − | + | + | + | + | − |
| **aqueous** | ***Enterococcus faecalis*** | − | − | − | − | + | + | + | + | − |
| **aqueous** | ***Salmonella typhi*** | − | − | − | − | − | + | + | + | − |
| **aqueous** | ***Enterobacter aerogenes*** | − | − | − | − | − | − | + | + | − |

- +: Positive inhibition
- - : Negative inhibition

**Table 8**: Minimum Bacterial Concentration (MBC) of ethanolic and aqueous *Rosmarinus officinalis* L. extract on *Listeria monocytogenes*, *Bacillus cereus*, *Enterococcus faecalis*, *Salmonella typhi* and *Enterobacter aerogenes*

| | | | | ***Rosmarinus officinalis* L. extract concentrations (mg/ml)** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Extract** | **Microorganism** | **2** | **4** | **8** | **16** | **32** | **64** | **128** | **256** | **control** |
| **ethanolic** | ***Listeria monocytogenes*** | − | − | − | + | + | + | + | + | − |
| **ethanolic** | ***Bacillus cereus*** | − | − | − | + | + | + | + | + | − |
| **ethanolic** | ***Enterococcus faecalis*** | − | − | − | − | − | + | + | + | − |
| **ethanolic** | ***Salmonella typhi*** | − | − | − | − | − | − | + | + | − |
| **ethanolic** | ***Enterobacter aerogenes*** | − | − | − | − | − | − | + | + | − |
| **aqueous** | ***Listeria monocytogenes*** | − | − | − | − | + | + | + | + | − |
| **aqueous** | ***Bacillus cereus*** | − | − | − | − | + | + | + | + | − |
| **aqueous** | ***Enterococcus faecalis*** | − | − | − | − | − | + | + | + | − |
| **aqueous** | ***Salmonella typhi*** | − | − | − | − | − | − | + | + | − |
| **aqueous** | ***Enterobacter aerogenes*** | − | − | − | − | − | − | − | + | − |

- +: Positive inhibition
- - : Negative inhibition

**DISCUSSION**

Based on the results ethanolic extract of *Lavandula stoechas* L. and *Rosmarinus officinalis* L. in this study have significant antimicrobial activity on the studied microorganisms. Antimicrobial effect of the extracts was different, depending on the type of microorganisms, thus, the gram-positive bacterium *Listeria monocytogenes*, *Bacillus cereus* and *Enterococcus faecalis*, was higher sensitivity compared to gram-negative bacteria *Salmonella typhi* and *Enterobacter aerogenes* (Table 3 and 4) and showed inhibitory effects at lower concentrations of *Lavandula stoechas* L. and *Rosmarinus officinalis* L. extracts. In many studies, the mechanism of the cell wall is considered. They have reported that cell wall and cell membrane affected and changed their permeability cause Release of intracellular contents, which can be associated with impaired membrane function, such as electron transfer, enzyme activity or nutrient uptake [20].

Ethanolic extract of *Rosmarinus officinalis* L. was more effective extract *Lavandula stoechas* L. (Table 3 and 4).

Bendini *et al*, (2002) reported that ethanolic extracts under selected conditions showed antioxidant activity [21]. In other spices, such as rosemary, antioxidant activity has been attributed to phenolic compounds like carnosic acid, rosmanol and rosmarinic acid so as to flavonoids. Phenolic compounds and flavonoids such as luteolin, hispidulin, apigenin, acacetin, diosmetin, herbacetin, quercetin, naringin, among others, had also been described in oregano extracts. Other compounds as, for example, rosmarinic acid have also been identified in oregano. Even though a variety of flavonoids are known there is no correlation between compositional data and antioxidant activity. Also, ethanolic extract compared to the aqueous extract was more effective and has a greater deterrent. The reason of these phenomena may be extracting more effective materials extracted by ethanol from *Lavandula stoechas* L. and *Rosmarinus officinalis* L.

The results indicated that the rosemary extracts showed antibacterial activity, according to mainly against the Gram-positive bacteria (*S. aureus* and *B. cereus*). The extracts also exhibited an effect against the Gram-negative bacteria (*E. coli* and *P. aeruginosa*) [22]. However, this effect was less efficient than that presented against the Gram-positive bacteria, since a higher MIC value was obtained with the Gram-negative bacteria. A similar behaviour was reported by Panizzi [23].

Therefore, using *Lavandula stoechas L.* and *Rosmarinus officinalis L.* as a natural antimicrobial compounds in vitro requires further research on mechanism of the pharmacy plant on the microorganisms.

Lavenders' antimicrobial activity is usually attributed to their terpenic compounds [24, 25]. In the essential oils of *stoechas* subsp. *luisieri*, the presence of necrodane derivatives have been reported as the characteristic and dominant compounds, but chemiotypes are mentioned [26, 27]. Recent studies also report a variable chemical composition in *L. pedunculata*, but concerning the presence of essential oils fenchone-, 1, 8-cineole- and camphor-rich [4].

Several mechanisms are discussed to explain the antimicrobial effect. Found that the antimicrobial compounds in the plant extract, have interaction with the phospholipids' two layers membrane, and affect the permeability of the bacterial cell membrane, and released the intracellular components [28].

The plants are a reserve of biologically active substances. Essential oils can be a significant source of a great diversity of chemical species equipped with antimicrobial capacity, the oil of Lavandula stoechas and Cistus ladaniferus can have application in therapy of the infectious diseases is like substituents of certain antibiotics or like complementary agents used in synergy with the synthesis substances. Essential oils can also have application in food industries not only like aromatizing but also like preservative of foodstuffs. In conclusion, it can suggest that *Lavandula stoechas* L.and *Rosmarinus officinalis* L. extract in "*in vitro*" have considerable antimicrobial ability over the studied strains. In addition, more studies are needed in "*in situ*" be done, to identifying the effective dose of the extract on the microorganisms, and finally introduce the extract as a natural and novel antimicrobial compound.

**ACKNOWLEDGMENT**

The authors wish to express their profound gratitude and thank sincerely to Research's Deputy of Ferdowsi University of Mashhad for providing the cost of this project and help to implementation of this project with code 2.29914 which adopted of the Research Council of Agricultural faculty.

**REFERENCES**

1. Peng, Y., Yuan, J., Liu, F., & Ye, J. Determination of active components in rosemary by capillary electrophoresis with electrochemical detection. Journal of Pharmaceutical and Biomedical Analysis. 2005; 39(3): 431-7.

2.Putnam, S. E., Scutt, A. M., Bicknell, K., Priestley, C. M., & Williamson, E. M. Natural products as alternative treatments for metabolic bone disorders and for maintenance of bone health. Phytotherapy Research. 2007; 21(2): 99-112.

3. Takahashi, T., Kokubo, R., & Sakaino, M. Antimicrobial activities of eucalyptus leaf extracts and flavonoids from Eucalyptus maculata. Letters in Applied Microbiology. 2004; 39(1): 60-4.

4. Zuzarte M, Dinis A M, Cavaleiro C, Salgueiro L, Canhoto JM. Trichomes, essential oils and in vitro propagation of Lavandula pedunculata (Lamiaceae). Ind. Crops Prod.2010; 32: 580-7.

5. Kim, J. T., Ren, C. J., Fielding, G. A., Pitti, A., Kasumi, T., Wajda, M., Bekker, A. Treatment with lavender aromatherapy in the post-anesthesia care unit reduces opioid requirements of morbidly obese patients undergoing laparoscopic adjustable gastric banding. Obesity Surgery. 2007; 17(7): 920-5.

6. Katona, J. M., Sovilj, V. J., & Petrović, L. B. Microencapsulation of oil by polymer mixture–ionic surfactant interaction induced coacervation. Carbohydrate Polymers. 2010; 79(3): 563-70.

7. Fernandez-Lopez, J., Zhi, N., Aleson-Carbonell, L., Perez-Alvarez, J., & Kuri, V. Antioxidant and antibacterial activities of natural extracts: application in beef meatballs. Meat Science. 2005; 69(3): 371-80.

8. Rahman, M., & Sh, G. Antibacterial activity of hydrodistilled essential oil of Psammogeton Canescens NO Umbelliferae. Biotechnology.2002; 1(1): 55-60.

9. Inouye, S., Takizawa, T., & Yamaguchi, H. Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. Journal of Antimicrobial Chemotherapy. 2001; 47(5): 565-73.

10. Ahmad, I., & Beg, A. Z. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. Journal of Ethnopharmacology. 2001; 74(2): 113-23.

11. Alizadeh Behbahani, B., Tabatabaei Yazdi, F., Shahidi, F., & Mohebbi, M. Antifungal effect of aqueous and ethanolic mangrove plant extract on pathogenic fungus "in vitro". International Journal of Agronomy and Plant Production. 2013; 4 (7): 1652-8.

12. Sattari M, Shahbazi N, Najjar Sh. The antibacterial activity of methanolic extract of Eucalyptus against Pseudomonas aeroginosa. Journal of Tarbiat Modarres. 2005; 8(1): 19-23. [In Persian].

13. Valero M, Salmeron M. (2003). Antimicrobial activity of 11 essential oils against Bacillus cereus in Tyndallized carrot broth. International Journal of Food Microbiology. 85, 73-81.

14. Collins, C. H., Lynes, P. M. and Grange, J.M. Microbiological Methods. (7$^{th}$Edn.) Butterwort-Heinemann Ltd., Britain.1995; pp.175-90.

15. Babayi, H., Kolo, I., Okogun, J., & Ijah, U. The antimicrobial activities of methanolic extracts of Eucalyptus camaldulensis and Terminaliacatappa against some pathogenic microorganisms. Biokemistri. 2004; 16(2): 106-11.

16. He F, Zhou J. A new antimicrobial susceptibility testing method of Escherichia coli against ampicillin by MSPQC. Journal Microbiological Methods. 2007; 68(3): 563-7.

17. Benger S, Townsend P, Ashford RL, Lambert P. An in vitro study to determine the minimum inhibitory concentration of Melaleuca alternifolia against the dermatophyte Trichophyton rubrum. The Foot. 2004; 14: 86 - 91.

18. Yazdani D RS, Amin Gh, Zainal Abidin MA ,Shahnazi S, Jamalifar H. Antifungal Activity of Dried Extracts of Anise (Pimpinella anisum L.) and Star anise (Illicium verum Hook. f.) Against Dermatophyte and Saprophyte Fungi. Journal of Medicinal Plants. 2009; 8(5): 24-9.

19. Espinel-Ingroff, A., Fothergill, A., Peter, J., Rinaldi, M., & Walsh, T. Testing conditions for determination of minimum fungicidal concentrations of new and established antifungal agents for Aspergillus spp.: NCCLS collaborative study. Journal of Clinical Microbiology. 2001; 40(9): 3204-8.

20.Tabatabaei Yazdi, F., Alizadeh Behbahani, B. Antimicrobial effect of the aqueous and ethanolic Teucrium polium L. extracts on gram positive and gram negative bacteria "in vitro".

Journal of Paramedical Science (JPS). 2013; 4(4), 55-61.
21.Bendini, A., Toschi, T. G., & Lercker, G. Antioxidant activity of oregano (Origanum vulgare L.) leaves. Italian Journal of Food Science. 2002; 14(1): 17-24.
22.Weckesser, S. et al. Screening of plant extracts for antimicrobial activity against bacteria and yeasts with dermatological relevance. Phytomedicine. 2007; 14(7): 508-16.
23.Panizzi, L. Composition and antimicrobial properties of essential oils of four Mediterranean Lamiaceae. Journal of Ethnopharmacology. 1993; 39(3): 167-170.
24.Lis-Balchin M, Deans SG, Eaglesham E. Relationship between bioactivity and chemical composition of commercial essential oils. Flavour Fragr Journal. 1998; 13(1): 98-104.
25.Hammer, K. A., Carson, C., & Riley, T. Antimicrobial activity of essential oils and other plant extracts. Journal of Applied Microbiology. 2001; 86(6): 985-90.
26.Matos F, Miguel M, Duarte J, Venancio F, Moiteiro C, Correia AI, Figueiredo AC, Barroso JG, Pedro LG . Antioxidant capacity of the essential oils from Lavandula luisieri, L. stoechas ssp. lusitanica, L. stoechas ssp. lusitanica x L. luisieri and L. viridis grown in Algarve (Portugal). J. Essent. Oil Res.2009; 21: 327-36.
27.González-Coloma, A., Delgado, F., Rodilla, J. M., Silva, L., Sanz, J., & Burillo, J. Chemical and biological profiles of Lavandula luisieri essential oils from western Iberia Peninsula populations. Biochemical Systematics and Ecology. 2011; 39(1): 1-8.
28.Kotzekidou P, Giannakidis P, Boulamatsis A. Antimicrobial activity of some plant extracts and essential oils against foodborne pathogens in vitro and on the fate of inoculated pathogens in chocolate. LWT-Food Science & Technology. 2008; 41(1): 119-27.